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Nonradiative Pathways of 7-Azaindole in Water

F. Gai, Y. Chen, and J. W. Petrich*

Contribution from the Department of Chemistry, Iowa State University, Ames, Iowa 50011.
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Abstract: The 7-azaindole chromophore in water is studied by means of picosecond absorption and fluorescence spectroscopy in order to determine its nonradiative decay pathways. It is concluded that a small population of 7-azaindole molecules (~20%) undergo excited-state tautomerization in about 70 ps. Intersystem crossing and photoionization are also identified as nonradiative decay channels. Photoionization occurs largely from an electronic state lying slightly in energy above the fluorescent state. This new understanding of the photophysics of the 7-azaindole chromophore in water will be essential in interpreting its behavior when it is used as an optical probe of protein structure and dynamics.

Introduction

7-Azaindole (7AI) is an important model system for both biochemical and physical chemical processes. It has attracted notice for its ability to execute excited-state proton transfer under certain conditions,[1-5] and it has been used as a probe of excited-state solvation dynamics.[4] Most recently, we have exploited it as the chromophoric moiety of the tryptophan analog, 7-azatryptophan (7AT),[5,6] a novel optical probe of protein structure and dynamics. 7AT can be incorporated into bacterial protein, it is amenable to peptide synthesis, and its absorption and fluorescence spectra are distinguishable from those of tryptophan.[5,6] Most important for its use as a probe, however, is that the fluorescence decay for 7AT over most of the pH range, when emission is collected over the entire band, is single exponential (780 ps), whereas for tryptophan a nonexponential decay is observed.[7]

An intriguing characteristic of the steady-state fluorescence of 7AI is that in alcohols one observes two bands with distinct maxima;[2-5] but in water, only a relatively smooth band is detected at neutral pH.[5] The redder of the two bands observed in alcohols is attributed to an excited-state "tautomer." Consequently, the bluer of the two bands is attributed to a "normal" species. Previously we suggested that the fluorescent species in water was predominantly "tautomer-like".[5] To explore this idea, it is necessary to detect and to characterize the photoproducts produced upon light absorption by the 7AI chromophore.

Experimental Section

Time-resolved absorption measurements[8] were performed with a system that will be described elsewhere. Fluorescence lifetimes were determined by a time-correlated single-photon counting apparatus[5] capable of resolving lifetimes as short as 20 ps when deconvolution of the instrument response is taken into account. TLC with silica gel plates and ethyl acetate indicates that commercial 7AI (Sigma) has an $R_f \sim 0.60$ and resolves two contaminants having $R_f \sim 0.15$ and 0.00. To remove the impurities, flash chromatography[9] was performed using ethyl acetate.

* To whom correspondence should be addressed.

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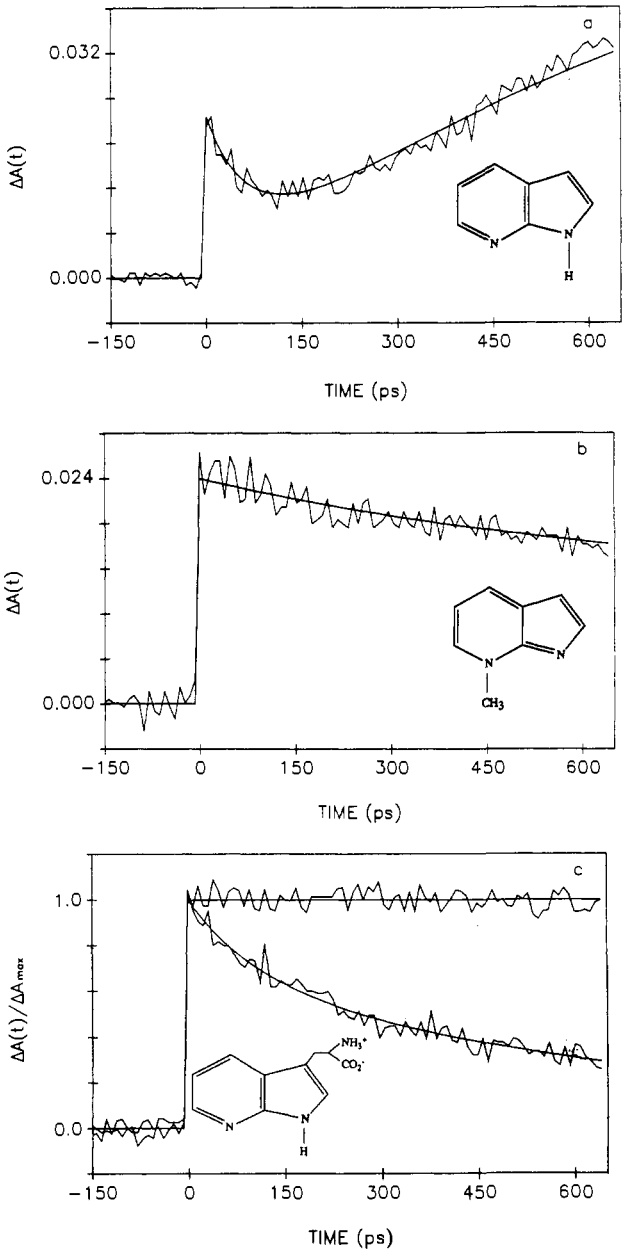

Figure 1. Transient absorption of 7AI and its analogs in water, 23 °C, λ_{ex} = 294 nm. (a) 7AI, pH 6.75, λ_{probe} = 392 nm. $\Delta A(t)$ = 0.022 exp($-t$/70 ps) + 0.065 [1 − exp($-t$/950 ps)]. Tuning the probe wavelength to the red diminishes the dip in absorption observed at 100 ps. The increased absorption toward the red is attributed to a solvated electron. (b) 7M7AI, pH 8.80, λ_{probe} = 392 nm. $\Delta A(t)$ = 0.012 exp($-t$/750 ps) + 0.012, where the constant represents a species that does not decay on the time scale of the experiment. (c) 7AT, pH 6.83, λ_{probe} = 580 nm. The two traces are normalized to have the same maximum absorption change. The lower of the two curves is obtained in the presence of 0.25 M KNO_3. The upper trace is flat on the time scale of the measurement. The maximum absorption change in the absence of scavenger is 0.16; in the presence of 0.25 M KNO_3, it is 0.05. Similar behavior is observed for 7AI, 1M7AI, and 7M7AI. Plots of the logarithm of ΔA_{max} against the logarithm of the pump intensity yield a straight line with a slope of 1.2 ± 0.3, indicating a monophotonic ionization.[13,18] Since the absorbance appears instantaneously on this time scale, we conclude[16] that the ionizing state is an upper electronic state.

The two impurities have nonexponential fluorescence lifetimes: $F(t)$ = 0.38 exp($-t$/607 ps) + 0.25 exp($-t$/2035 ps) + 0.37 exp($-t$/8895 ps) and $F(t)$ = 0.18 exp($-t$/383 ps) + 0.37 exp($-t$/1087 ps) + 0.45 exp($-t$/7414 ps). Samples are changed regularly. Subsequent to light exposure they are analyzed by TLC to monitor their integrity. Methylated derivatives were prepared that mimic untautomerized and tautomerized 7AI: N_1-methyl-7-azaindole (1M7AI), and 7-methyl-7*H*-pyrrolo[2,3-*b*]pyridine[10] (7M7AI).

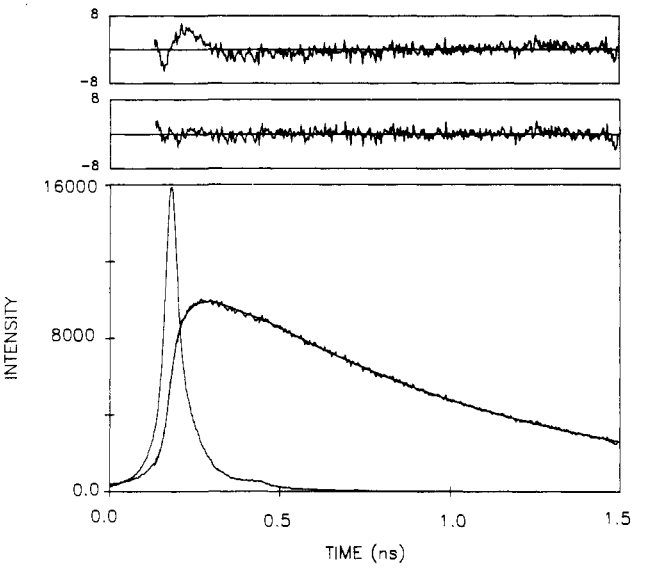

Figure 2. Fluorescence decay of 7AI in water, pH 6.1, 20 °C, λ_{ex} = 288 nm, λ_{em} = 380 nm (16-nm bandpass). The upper set of residuals corresponds to a single-exponential fit to the data, which yields a decay time of 816 ps with χ^2 = 2.6. The lower set of residuals corresponds to a double-exponential fit yielding $F(t)$ = 0.20 exp($-t$/41 ps) + 0.80 exp($-t$/835 ps), χ^2 = 1.2. In preliminary work[5] we could not resolve the short-lived component because experiments were performed on a 6-ns full-scale time base. Depending on whether a full-scale time base of 1.5 or 3.0 ns is used, the lifetime obtained for the short-lived component is either ~40 or ~100 ps, respectively. In either case, however, its amplitude remains the same.

Results and Discussion

Figure 1a presents the transient absorbance at 392 nm of 7AI in water at 23 °C. Two salient features are the instantaneous appearance of a species that undergoes a rapid ~70-ps decay and the slow growth of another species with a 950-ps rise time. We shall first comment on the 70-ps decay. A transient of this duration is consistent with data obtained from time-correlated single-photon counting experiments (Figure 2). Measurement of the fluorescence decay at wavelengths across the emission band up to ~400 nm reveals a small contribution of an ~40-ps decay component. The fluorescence decay component is matched by a corresponding fluorescence rise time at wavelengths ≥400 nm. We observe similar behavior in the emission of 7AT.

The fast decay component displayed in Figure 1a most likely arises from the absorption of the excited-state singlet of a "normal" species in water, which is depleted by tautomerization. This assignment for the rapid component in water is supported by the absence of analogous rapidly decaying or rising transients in the time-resolved fluorescence emission of 7M7AI and 1M7AI, which cannot tautomerize. The temperature dependence of the short-lived component in water yields an Arrhenius activation energy of 2.7 ± 1.8 kcal/mol, which is consistent with data for alcohols[3-6] that indicate that large-amplitude solvent motion is required for tautomerization. (A 40–100-ps decay is too long to be attributed to solvation dynamics in water, which occur on a time scale of ≤1 ps.[11]) There is no 70-ps component in the transient absorption of 7M7AI or 1M7AI. The shortest-lived component observed in absorption measurements of 7M7AI is 750 ps (Figure 1b), which agrees well with its fluorescence lifetime in water that we have measured; hence it is attributed to absorption by the excited-state singlet of 7M7AI.

Figure 1b reveals an absorbance that does not decay on the time scale of the measurement. This absorption may be present to a

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small extent in Figure 1a. If the probe wavelength is tuned farther to the red for 7AI in water, the 70-ps transient is obscured by this long-lived transient; at 450 nm the transient is no longer observable, presumably because the extinction coefficient for the long-lived species increases toward redder wavelengths. To the red of 580 nm, the 950-ps rise time is also obscured by the long-lived species. On the basis of the data displayed in Figure 1c, we attribute this species to a solvated electron, e^-_{aq}. The long-lived absorption that we detect with the white-light continuum from our picosecond absorption apparatus from 400 to 720 nm is consistent with published spectra for e^-_{aq}.[12,13] This assignment is strengthened by the observation that the electron scavenger,[14] KNO_3, transiently quenches this absorbing species. Collins suggested the possibility of photoinduced electron ejection in excited-state complexes of 7AI and solvent.[15] We provide the first direct evidence for the production of e^-_{aq}. Because the rise time of the electron appearance is instantaneous on the time scale of our experiment, we propose that the origin of e^-_{aq} is an electronic state lying slightly above the fluorescent state. This assignment is strengthened by our observation of two overlapping electronic states in the fluorescence-excitation anisotropy spectrum of 7AI.[16]

Finally, the 950-ps rise time for the transient absorbance at 392 nm of 7AI in water agrees, within experimental error, with the 915-ps fluorescence decay of 7AI in water.[11] There is evidence[17] for triplet formation in 7AI, and we tentatively assign the rising absorption at 392 nm to a triplet species. Bent and Hayon observe triplet absorption for indole and tryptophan at similar wavelengths.[13]

Conclusions

Our earlier hypothesis concerning the "tautomer-like" nature of the fluorescent species requires modification. The 70-ps decay of the excited-state absorbance of 7AI in water (Figure 1a) and the similar decay and rise times on the blue and red edges of the 7AI emission spectrum (Figure 2) indicate that *at least a fraction* of the 7AI in aqueous solution is capable of executing excited-state tautomerization. This suggests that the observed single exponential decay of the 7AI chromophore in water, when monitoring the *entire* emission band, is a fortuitous consequence of compensating the small contributions of decaying and rising lifetime components. We have also detected additional products of nonradiative decay from the 7AI excited state: the solvated electron and a species that is assigned to a triplet. In order to unravel the photophysics of 7AI and 7AT and to understand why 7AT appears to be such a well-behaved probe molecule, it will be necessary to take these nonradiative channels into account.

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One-Electron Reduction of Methyl(trifluoromethyl)dioxirane by Iodide Ion. Evidence for an Electron-Transfer Chain Reaction Mediated by the Superoxide Ion

Waldemar Adam,*,† Gregorio Asensio,*,‡ Ruggero Curci,*,§ Maria Elena González-Núñez,‡ and Rossella Mello‡

Contribution from the Institut für Organische Chemie, Universität Würzburg, D-8700 Würzburg, Germany, Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Valencia, Avda. Blasco Ibáñez 13, E-46010 Valencia, Spain, and Centro CNR "MISO", Dipartimento di Chimica, Università di Bari, Via Amendola, 173, I-70126 Bari, Italy. *Received April 3, 1992*

Abstract: One-electron-transfer processes, triggered by I^- in catalytic amounts, convert methyl(trifluoromethyl)dioxirane (**1**) into 1,1,1-trifluoropropanone (trifluoroacetone) and dioxygen. It is proposed that the initially formed bis(oxy)methylene radical anion **1'** performs nucleophilic attack at the dioxirane **1**, yielding a dimeric radical anion **2'**; the latter then fragments into trifluoroacetone and superoxide ion ($O_2^{\bullet-}$). The intermediacy of superoxide ion (the propagator of the electron-transfer chain reaction) is demonstrated by its trapping with either benzoyl chloride or chlorotrimethylsilane. Also, potassium superoxide in catalytic amounts induces the fast and complete conversion of **1** into trifluoroacetone with evolution of dioxygen. The redox reaction between dioxirane **1** and iodide ion in acidic medium yields substantial amounts of hydrogen peroxide, which was detected by the catalase probe.

Introduction

Methyl(trifluoromethyl)dioxirane (**1**), obtained[1] in a solution of 1,1,1-trifluoropropanone (hereafter trifluoroacetone) by reaction of the latter ketone with potassium peroxomonosulfate (Caroate, the triple salt $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$), is one of the most powerful yet selective oxygen-transfer reagents toward a variety of substrates.[2] During the last two years, new aspects concerning

† Universität Würzburg.
‡ Universidad de Valencia.
§ Università di Bari.

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