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Novel Ground- and Excited-State Prototropic Reactivity of a Hydroxycarboxyflavylium Salt

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Synthetic and natural hydroxyflavylium salts are super-photoacids, exhibiting values of the rate constant for proton transfer to water in the excited state as high as 1.5 × 10^{11} s^{-1}. The synthetic flavylium salt 4-carboxy-7-hydroxy-4′-methoxyflavylium chloride (CHMF) has an additional carboxyl group at the 4-position of the flavylium cation that deprotonates in the ground state at a lower pH ($pK_{a1} = 0.73$; $AH_2^+ \rightarrow Z$) than the 7-hydroxy group ($pK_{a2} = 4.84$; $Z \rightarrow A^-$). Ground-state deprotonation of the carboxyl group of the acid (AH_2^+) to form the zwitterion (Z) is too fast to be detected by nanosecond laser flash perturbation of the ground-state equilibrium, while deprotonation of the hydroxyl group of Z to form the anionic base (A^-) occurs in the microsecond time range ($k_{d2} = 0.6 \times 10^6$ s^{-1} and $k_{p2} = 4.2 \times 10^{10}$ $M^{-1}{\cdot}s^{-1}$). In the excited state, the cationic form (AH_2^{+}*) deprotonates in ∼9 ps, resulting in the excited neutral base form (AH*), which is unstable in the ground state. Deprotonation of Z* occurs in 30 ps ($k_{d2}{}^* = 2.9 \times 10^{10}$ s^{-1}), to form excited A^-*, which either reprotonates ($k_{p3}{}^* = 3.7 \times 10^{10}$ $M^{-1}{\cdot}s^{-1}$) or decays in 149 ps, and shows an important contribution from geminate recombination to give the excited neutral base (AH*). Predominant reprotonation of A^-* at the carboxylate group reflects both the presence of the negative charge on the carboxylate and the increase in the excited-state pK_a of the carboxyl group. Thus, while the hydroxyl pK_a decreases by ∼5 units upon going from the ground state ($pK_a = 4.84$) to the excited state ($pK_a{}^* = -0.2$), that of the carboxyl group increases by at least this much. Consequently, the excited state of the Z* form of CHMF acts as a molecular proton transporter in the picosecond time range.

Introduction

Synthetic and natural hydroxyflavylium salts are super-photoacids, displaying values of the rate constant for proton transfer to water in the excited state as high as 1.5 × 10^{11} s^{-1}.[1−3] The fast deprotonation of hydroxyflavylium cations (in 6−20 ps), together with the short excited-state lifetime of the corresponding conjugated bases (130−250 ps), permits rapid (in ∼250 ps) displacement of the ground-state acid−base equilibrium toward the base by excitation of the flavylium cation with a nanosecond laser pulse.[4−6] The recovery to equilibrium after the *light jump* can then be followed, making the determination of ground-state prototropic reaction rates in the 10^8−10^4 s^{-1} range possible, which is not possible with conventional stopped flow or temperature jump techniques.[4−6]

All natural hydroxyflavylium salts (anthocyanins) undergo three additional types of reaction in aqueous solution: (1) hydration of the flavylium cation (AH^+), (2) tautomerization of the hemiacetal (B), and (3) isomerization of the *Z*-chalcone to the *E*-chalcone (Scheme 1). [7−10]

Both the deprotonation and hydration rate constants of flavylium cations diminish substantially in sodium dodecyl sulfate (SDS) micelles as a result of electrostatic stabilization of the positively charged AH^+, with respect to the neutral forms A and B, by the negative charge of the SDS micelle.[5,6,11] Because stabilization of the red color of AH^+ is highly desirable in connection with the use of anthocyanins as food dyes,[12−14] the SDS results provided a framework for interpreting the extent of intramolecular stabilization of AH^+ that might result from the introduction of an appropriate negatively charged substituent. The synthetic flavylium salt 4-carboxy-7-hydroxy-4′-methoxyflavylium chloride (CHMF, Scheme 2) possesses a carboxyl group at the 4-position of the flavylium cation, which is expected to deprotonate at pH values lower than the hydroxyl pK_a. In this work, we present a detailed analysis of the coupled

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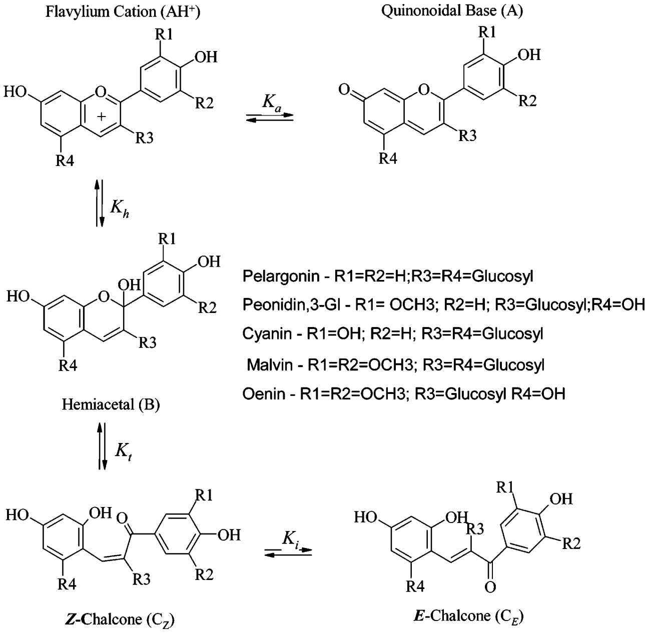

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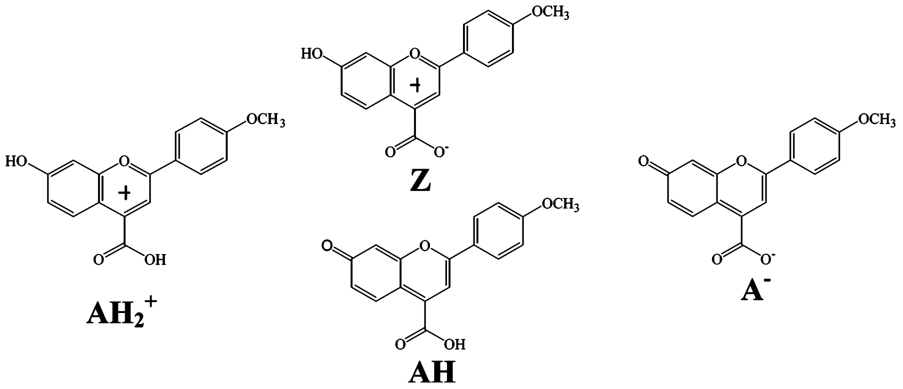
SCHEME 2



prototropic reactions exhibited by this compound, in both the ground and excited states.

Experimental Section

Materials. The synthetic flavylium salt 4-carboxy-7-hydroxy-4′-methoxyflavylium chloride (CHMF), a known compound,[14,15] was a generous gift from Prof. Raymond Brouillard (Université Louis Pasteur, Strasbourg, France). Sodium phosphate and chloride, p.a., from Sigma were used without further purification in the equilibrium measurements. Ultrapure bioreagent grade sodium phosphate, hydrogen phosphate, and borate (Merck) and bis-Tris (Aldrich) were employed in the kinetic measurements.

Sample Preparation. Aqueous solutions were prepared in Millipore Milli-Q quality water. The concentration of CHMF was $\sim 10^{-5}$ M. Sodium phosphate buffer (10 mM) was employed to maintain the required pH. The final pH was measured with a Crison micropH 2002 or an ORION 720A pH meter with a combined RedRod electrode. Below pH 2, the desired hydrogen ion concentration was obtained by exact dilution of commercial perchloric acid (Riedel-de-Haen, PA 60%).

Absorption and Fluorescence Measurements. UV−vis absorption spectra were recorded on a Beckman DU-70, a Hewlett-Packard 8452A diode array, or a Shimadzu UV-2510 PC UV−vis recording spectrophotometer. Steady-state fluorescence spectra were recorded on a SPEX F212l Fluorolog or a SPEX-Jobyn Yvon Fluorolog 3 spectrofluorimeter. All fluorescence spectra are corrected. Fluorescence quantum yields were obtained using the fluorescein dianion in 0.01 M aqueous NaOH ($\phi_f = 0.90$)[16] as standard.

Laser Flash Photolysis. The laser flash photolysis experiments were carried out with an Edinburgh Analytical Instruments LP900 laser flash photolysis system as previously described.[4] Excitation was carried out with the third harmonic (355 nm) of a Surelite II-10 Nd:YAG laser. Solutions were stirred between each laser shot and 10 laser shots averaged to obtain the transient absorption decays. The decays were analyzed using the Edinburgh Analytical Instruments LP900 system software. Lifetimes shorter than 30 ns were deconvoluted using the pulse shape of the laser (obtained by monitoring the Raman scattering peak of water in the fluorescence mode of the LP900). Since the transient lifetime was wavelength independent, transient absorption spectra were obtained by plotting the pre-exponential coefficients of the decays (or recoveries) at each wavelength.

Time-Resolved Fluorescence. Fluorescence decays were measured using the time-correlated single photon counting technique as described elsewhere.[3] Alternate measurements (10^3 counts at the maximum per cycle) of the excitation pulse profile and sample emissions were made until typically 3×10^3 total counts had been accumulated at the maximum. Fluorescence decays were deconvoluted from the excitation pulse by using G. Striker's Sand program, which permits individual and global analysis of the decays with individual shift optimization.[17]

Molecular Orbital Calculations. Ab initio calculations were performed at the density functional theory (DFT) level as implemented in the Gaussian 03 package.[18] Gas-phase single point energies were obtained by using the mPW1PW91[19] functional and a 6-311+G(2d,2p) basis set with mPW1PW91/6-31+G(d,p) optimized geometries. Energy minima were confirmed by harmonic frequency calculations at the mPWIPW91/6-31+G(d,p) level. The translational, rotational, and vibrational contributions to the gas-phase enthalpies of the molecules were calculated within the framework of statistical thermodynamics.[20] Geometries were also optimized in aqueous solution at the mPW1PW91/6-31G(d) level using an implicit solvent described by the integral equation formalism for the polarizable continuum model[21] (IEFPCM). Solvation free energies were computed at the HF/6-31G(d) level, employing the united atom model for Hartree−Fock[22] (UAHF) to build the molecular cavity. The absorption wavelengths and oscillator strengths were calculated by time-dependent DFT (TD-DFT) and ZINDO for the optimum geometries obtained in aqueous solution with mPW1PW91/6-31+G(d) and the IEFPCM.

Results

Multiequilibria of CHMF. Figure 1a shows absorption spectra of 4-carboxy-7-hydroxy-4′-methoxyflavylium chloride (CHMF) in aqueous solution at 20 °C at pH values within the range 2.12−7.17 taken immediately (3 s) after sample preparation.[5] The isosbestic wavelength at 478 nm is characteristic of an acid−base equilibrium involving only two species within this pH range: below pH ∼4.5, an acid form with a maximum absorbance at 458 nm, and, above pH 4.5, a base form with a maximum absorbance at 494 nm. Upon standing, the absorption spectrum of these solutions gradually changes over a period of ∼1 h, with a decrease in the absorption at 458 and 494 nm (∼20%) and an increase in absorbance at 360 and 280 nm. Such spectral changes are characteristic of the occurrence of the classical multiequilibria of anthocyanins, the time-dependent absorbance changes corresponding to the formation of the hemiacetal (B) (at 275 nm) and the chalcones (300−400 nm) at the expense of the acid and base forms.[5]

Decreasing the pH below pH 2 induces the appearance of a third species with a maximum absorbance at 484 nm and a second isosbestic wavelength at 474 nm, indicating an additional acid−base equilibrium (Figure 1b). The absorption band at 484 nm is thus assigned to the flavylium cation (AH_2^+), which is present only under strongly acidic conditions (pH < 1.0). The

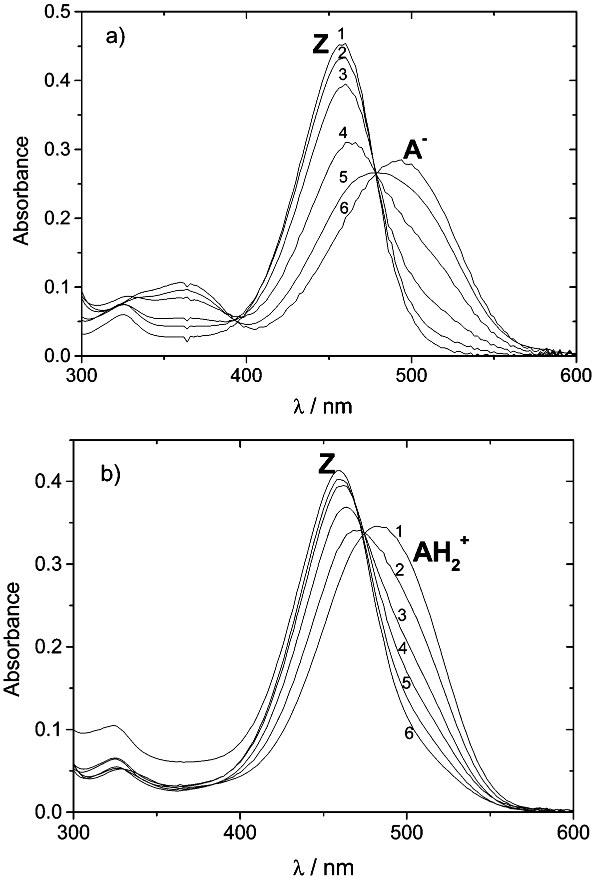


Figure 1. Absorption spectra of CHMF in (a) buffered aqueous solutions at pH 2.12 (1), 3.32 (2), 3.90 (3), 4.48 (4), 5.08 (5), and 7.17 (6) (the isosbestic wavelength of the Z and A^- forms is at 478 nm) and (b) acidified ($HClO_4$) aqueous solutions at pH -0.54 (1), -0.07 (2), 0.43 (3), 0.94 (4), 1.43 (5), and 1.99 (6) (the isosbestic wavelength of AH_2^+ and Z is at 474 nm).

absorption at 458 nm can be due to a quinonoidal base (AH) or to a zwitterionic species resulting from deprotonation of either the hydroxyl (Z) or the carboxyl group (AH). Finally, the absorption band at 494 nm is assigned to the ionized quinonoidal base (A^-) (Scheme 2).

$$AH_2^+ \underset{k_{p1}[H^+]}{\overset{k_{d1}}{\rightleftharpoons}} AH/Z + H^+ \underset{k_{p2}[H^+]}{\overset{k_{d2}}{\rightleftharpoons}} A^- + 2H^+$$

The mole fractions of AH^+, AH/Z, and A^-, evaluated by decomposition of the spectra in Figure 1 taken immediately (3 s) after dissolution of CHMF, are plotted as a function of the pH in Figure 2. Values of $pK_1 = 0.73 \pm 0.13$ and $pK_2 = 4.84 \pm 0.20$ at 20 °C were obtained from nonlinear fitting of the mole fractions to the appropriate equations.[23] The value of pK_2 is in reasonable agreement with a previously reported value at 25 °C (4.78).[14] This value is only slightly larger than the typical range of pK_a values of synthetic or natural flavylium salts (e.g., 3.7 for oenin and 4.4 for 7-hydroxy-4-methylflavylium chloride),[1] which refer to deprotonation of the AH^+ cation to give the neutral base (A) rather than deprotonation of a neutral species (such as Z) to give an anion (A^-), as in the case of CHMF. The cationic flavylium ion form of CHMF (AH_2^+), on the other hand, is extremely acidic ($pK_{a1} = 0.73$). Interestingly, the absorption maxima of the AH/Z (458 nm) and A^- (494 nm) forms of CHMF are remarkably similar to those of the AH^+ (458 nm) and A (495 nm) forms of the parent compounds 4′,7-dihydroxyflavylium chloride (DHF) and 4′,7-dimethoxyflavylium chloride (DMF).[10,24]

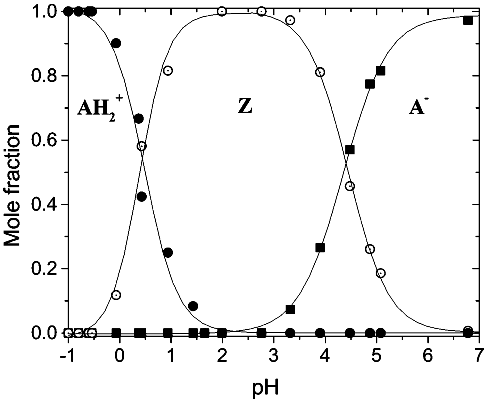


Figure 2. Mole fractions of the AH_2^+ (●), Z (⊙), and A^- (■) forms of CHMF in nonequilibrated solutions as a function of the pH; $pK_{a1} = 0.73$ and $pK_{a2} = 4.84$.

TABLE 1: Experimental (λ_{exptl}) and Theoretical (λ_{theor}) Wavelengths of the S_1-S_0 Electronic Transitions in the Absorption Spectra, Electronic Enthalpy in the Ground State Relative to the AH_2^+ Form ($\Delta H_{electron}$), Standard Gibbs Free Energies of Solvation in Water (ΔG_{solv}), and Dihedral Angles between the Flavylium B-Ring and the Carboxyl Group

	λ_{theor} (nm) TD-DFT	λ_{theor} (nm) ZINDO	λ_{exptl} (nm)	$\Delta H_{electron}$ (kcal/mol)	ΔG_{solv} (kcal/mol)	$\theta_{ring/COOH}$
AH_2^+	459	481	484	0	−51	35.4
Z	421	424	458	−19	−32	47.9
AH	509	509		−18	−18	29.5
A^-	459	472	494	−31	−63	40.6

MO Calculations and the Nature of the AH/Z Form. As previously mentioned, there are a priori two possible products of deprotonation of the flavylium form (AH_2^+) of CHMF: the quinonoidal base (AH) or its zwitterionic form (Z), resulting respectively from deprotonation of the hydroxyl or carboxyl group (Scheme 2). Table 1 shows experimental (λ_{exptl}) and theoretical (λ_{theor}) wavelengths of the S_1-S_0 electronic transitions of the four possible forms of CHMF. Comparison of a number of methods for calculation of electronic transitions in anthocyanins[25] has shown that the TD-DFT underestimates the transition wavelengths by ~30 nm for all species, while ZINDO/S correctly predicts the absorption spectrum of flavylium cations but underestimates the transition wavelength of the bases by ~25−30 nm. The results shown in Table 1 indicate that the absorption band at 458 nm is due to the zwitterionic form arising from deprotonation of the carboxyl group.

The calculated electronic enthalpies ($\Delta H_{electron}$) of AH and Z are nearly identical, but the standard Gibbs free energy of solvation (ΔG_{solv}) of Z is significantly more negative, making Z more stable than AH. We thus assign the absorption at 458 nm to the Z form.

Finally, the large dihedral angle between the carboxyl group and the plane of the pyrilium ring is consistent with the negligible effect of the carboxyl group on the absorption spectra of the AH/Z (458 nm) and A^- (494 nm) forms of CHMF relative to those of the AH^+ (458 nm) and A (495 nm) forms of the parent compounds DHF and DMF.[24] Thus, the zwitterion is in fact a flavylium cation with a nonresonant, negatively charged group at the 4-position.

Steady-State and Time-Resolved Fluorescence. Fluorescence spectra of CHMF in water at 20 °C, at pH values in the 0−1.25 range, resulting from excitation at the isosbestic wavelength (474 nm), are presented in Figure 3a. At pH ~0, the spectrum is that of the $AH_2^{+}*$ cation, with maximum

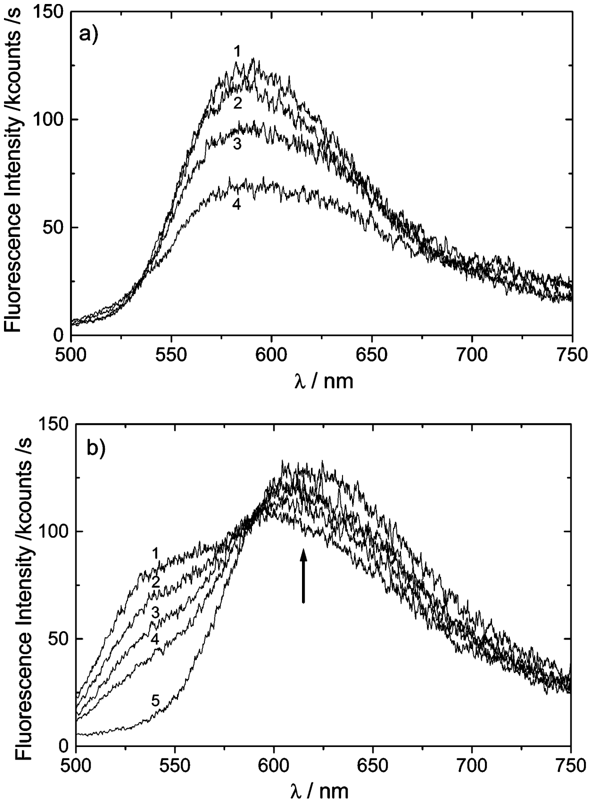


Figure 3. Fluorescence spectra of CHMF in (a) acidified ($HClO_4$) aqueous solutions at pH 0 (1), 0.30 (2), 1.14 (3), and 1.25 (4), measured with excitation at 474 nm, and (b) buffered aqueous solutions at pH 3.09 (1), 4.25 (2), 4.72 (3), 5.64 (4), and 6.37 (5) (λ_{exc} = 478 nm).

intensity at 585 nm. Upon an increase of the pH up to 1.25, the emission of Z* at 530 nm starts to develop at the expense of the emission of AH_2^{+}* (isoemissive wavelength at ~536 nm). Above pH 3, a second isoemissive wavelength of Z* and A^-*, at 584 nm, is observed (Figure 3b). Finally, above pH 6.7, only the emission of A^-* (620 nm) is observed.

At pH 7.2, the fluorescence decay of CHMF in water (λ_{exc} = 445 nm), measured at 650 nm (emission of A^-*), is single exponential. The lifetime of the base form (149 ps) is within the range observed for other synthetic and natural anthocyanins (130−250 ps).[1,5]

Within the pH range 2−3, where only the Z form exists in the ground state (see Figure 2), the fluorescence decay at 525 nm (emission of Z*) is a single exponential with a decay time of 31 ps. In contrast, at 650 nm (A^-* emission), double exponential decay is observed with a rise time (negative pre-exponential coefficient A_{22}) of around 20 ps, systematically smaller than the decay time at 525 nm. At pH values within the pH range 1.22−2 (where the mole fraction of Z is always greater than 0.95), the decay of Z* at 525 nm becomes double exponential, due to the appearance of an additional long decay time similar in magnitude to the long decay time observed at 650 nm. Although there is some variation in the values of the long lifetime derived from individual analyses at the two wavelengths, global analysis of the decays provides acceptable double exponential fits (Figure 4). Although triple exponential fits with two short decay times (~20 and ~30 ps) and one long decay time are better, the differences do not justify a claim for triple exponential decays at the present stage of the data. However, a small contribution of a third species in the fluorescence decays is possible (see Scheme 4).

The pattern of decay times and pre-exponential coefficients obtained from global double exponential analysis as a function of pH (Table 2) is typical of excited-state proton transfer,[1] except for the fact that the pre-exponential coefficient (A_{11}) of the long decay time (τ_1) of Z* at 525 nm is experimentally zero (corresponding to an equal quality of single and double exponential fits in the individual analysis) down to pH 2. At this pH, protonation of A^-* would normally result in a significant contribution (nonzero A_{11}) from the long decay time of Z* at 525 nm (see the Discussion section).

At pH values below 0.7, where the cation AH_2^{+} becomes the dominant form, the decays measured close to the maximum (580 nm) and on the tail (650 nm) of the 585 nm emission band show a rise time of ~9 ps followed by a decay time in the range of 40 ps. The presence of the rise time indicates that the emission arises (either exclusively or predominantly) from a species that is formed in the excited state at the expense of AH_2^{+}*. The decays measured at the onset (555 nm) of the emission band exhibit the same two times plus a residual contribution from the emission of Z*, but at this wavelength, the 9 ps time appears as a decay time (positive pre-exponential coefficient). The results indicate that the emission band with a maximum at 585 nm is, in fact, due to the strong overlap of the emission of AH_2^{+}* with that of a second species that is not Z*. This second species can only be AH*, formed by preferential deprotonation of the hydroxyl group of AH_2^{+}* (deprotonation of the carboxyl group being disfavored in the excited state). An exact analysis of these decays is not straightforward due to the strong overlap of the emission bands, the absence of AH as a stable species in the ground state, and, most importantly, the problems arising from the use of very high $[H^+]$ concentrations, in the range of ~1 M. In any event, however, the 9 ps time should provide a good estimate for the rate of deprotonation of AH_2^{+}* ($k_{d1}^* \approx 1.1 \times 10^{11}$ s^{-1}).

Ground-State Prototropic Reaction Kinetics. Figure 5a shows the transient absorption spectrum of CHMF in aqueous solution at pH 3.85, at $t = 0$, upon excitation with a 5 ns laser pulse at 355 nm. The spectrum shows the depletion of Z at 455 nm and an absorption band with a maximum at 505 nm that matches the absorption spectrum of A^-. The depletion of Z at 455 nm and the absorption of A^- at 505 nm demonstrate that excitation of Z leads to deprotonation to form the base A^-, as found for a variety of flavylium salts (see the Discussion section).[4−6] At both wavelengths, the transients decay with first-order kinetics and identical decay times (Figure 5b). Figure 6 shows a plot of the reciprocal decay time (the observed rate constant), which is a linear function of proton concentration. In the pH range where significant amounts of AH_2^{+} are present in solution (pH < 0.7), any transients would decay too quickly (<3 ns) to be detected with our instrumentation.

Discussion

Electronic Absorption and Emission of CHMF. The first interesting observation made with CHMF is the strong increase in the acidity of the carboxyl group attached to the flavylium moiety ($pK_{a1} = 0.73$, as compared with the value $pK_a = 4.20$ of benzoic acid). This implies substantial resonance, with charge transfer from the carboxyl to the flavylium ion, despite the nonzero dihedral angle ($\theta = 35°$) of the carboxyl group relative to the flavylium B-ring in the AH_2^{+} form (Table 1). The carboxyl−flavylium resonance is in fact reflected in the strongly red-shifted absorption spectrum of the AH_2^{+} form (λ_{max} = 484 nm in aqueous solution; Figure 1b) relative to that of the AH^+ form of the parent compound 4′,7-dihydroxyflavylium chloride (DHF; λ_{max} = 458 nm in aqueous solution).

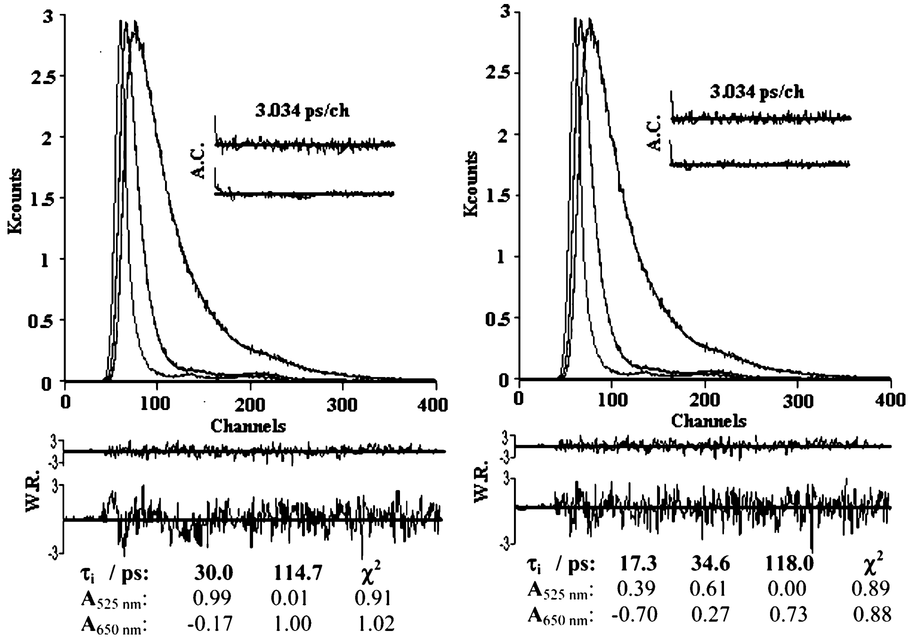
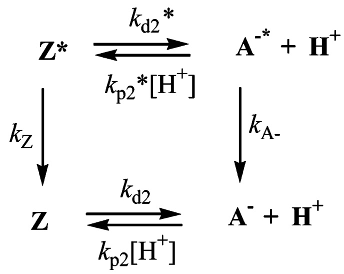


Figure 4. Global analysis of fluorescence decays of CHMF in H_2O at pH 2.00, $\lambda_{exc} = 445$ nm and $\lambda_{em} = 525$ and 650 nm (the decay at 525 nm is quasi-single exponential).

SCHEME 3



SCHEME 4

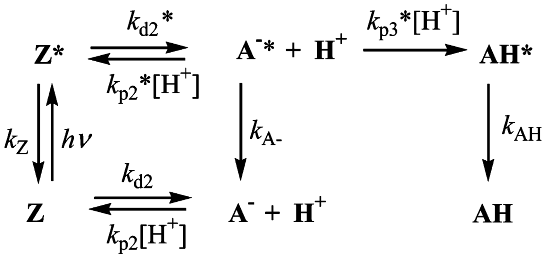


TABLE 2: Decay Times (τ_i) and Pre-exponential Coefficients at 525 nm (A_{1j}) and 650 nm (A_{2j}) from Global Analysis of CHMF Decays in Aqueous Solution at Different pH Values

pH	τ_1 (ps)	τ_2 (ps)	A_{11}	A_{12}	A_{21}	A_{22}
2.88	121	32.5	−0.001	1.000	1.00	−0.22
2.75	119	31.6	0.001	0.999	1.00	−0.21
2.26	115	33.4	−0.002	1.000	1.00	−0.30
2.06	113	33.1	−0.004	1.000	1.00	−0.30
2.00	115	30.0	0.004	0.996	1.00	−0.17
1.93	115	31.9	0.005	0.995	1.00	−0.28
1.69	110	31.9	0.006	0.994	1.00	−0.39
1.50	101	30.0	0.010	0.990	1.00	−0.27
1.22	100	29.7	0.015	0.985	1.00	−0.31

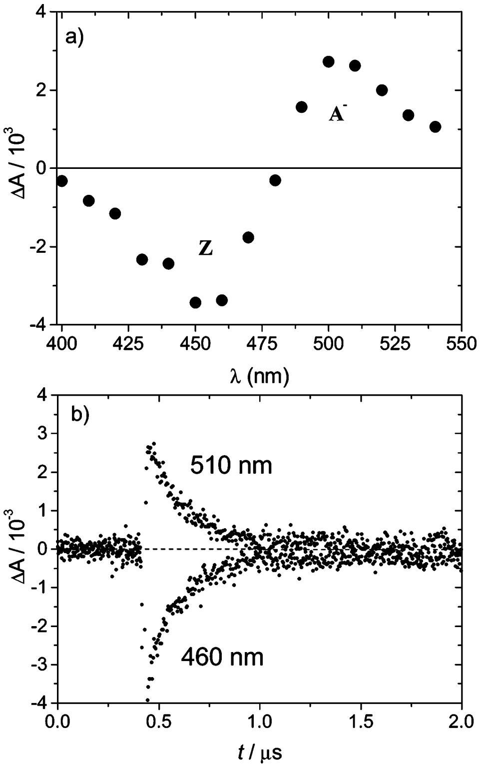


Figure 5. Transient absorptionof CHMF in aqueous solution at pH 3.85 resulting from excitation with a 5 ns laser pulse at 355 nm: (a) decay of A^- at 505 nm and recovery of Z at 455 nm; (b) transient spectrum measured immediately after the laser pulse.

The calculated dihedral angle (θ) is larger in the zwitterion form (Z) (48°), reducing the carboxylate−flavylium π-orbital overlap. This is apparently sufficient to make the absorption spectra of the Z form of CHMF and the AH^+ form of DHF similar to each other. The similarity between the pK_{a2} value of CHMF (4.84) and the pK_a value of DHF (4.0)[10] further indicates that, despite being a zwitterion, Z exhibits properties typical of a flavylium cation.

Although the zwitterion form (Z) of CHMF does hydrate in water, it does so to a smaller extent than either of the parent compounds DHF[10] or DMF.[24] Thus, in contrast to the strong stabilizing effect of anionic SDS micelles observed for all flavylium salts studied to date,[5,6,11] the presence of a carboxylate

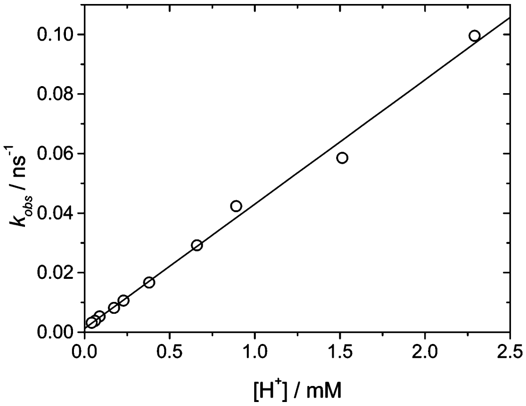


Figure 6. Plot of the observed rate constant for return to the ground-state Z−A^- equilibrium of CHMF as a function of proton concentration.

group in CHMF does not appreciably stabilize Z with respect to the hemiacetal form (B).

Ground-State Kinetics. The fast deprotonation of ordinary flavylium cations in the excited state (6−30 ps) and the short lifetime of the corresponding excited conjugated bases (130−250 ps) permit displacement of the ground-state acid−base equilibrium toward the base (A) in ~250 ps, upon excitation of the flavylium cation with a nanosecond laser pulse.[4−6] Subsequently, the excess of ground-state base protonates, returning the system to equilibrium. The kinetics of this process can be followed by monitoring the transient absorbance of either the depleted flavylium (recovery of AH^+) or the excess of base (decay of A). Both transients normally obey first-order kinetics with identical rates, $k_{obsd} = k_d + k_p[H^+]$.[4] Typically, k_d is of the order of 10^6 s^{-1} and k_p is diffusion controlled ((2.2−3.6) × 10^{10} M^{-1}·s^{-1}).[2]

In the case of CHMF, all excited species decay in the picosecond time range, well within the width of the nanosecond laser pulse. In the low pH range (<0.7), where AH_2^+ is the dominant form, no transients are observed. Indeed, at such low pH values, the return to equilibrium in the ground state should be much faster than the time resolution of our nanosecond laser flash photolysis system (~3 ns). Thus, since $pK_{a1} = 0.73$, the assumption of diffusion-controlled protonation (k_p ~(2.2−3.6) × 10^{10} M^{-1}·s^{-1})[2,4] would predict that k_{d1} is at least 7 × 10^9 s^{-1}. Hence, k_{obsd} would be expected to be of the order of ~10^{10} s^{-1} and the decay of any transients much too fast (<1 ns) to be resolved by our system.

In the 1.7−4 pH range, the zwitterion (Z) is the dominant form and its excitation leads to deprotonation of the hydroxyl group to form the excited base (A^-*) (see the fluorescence spectra in Figure 3b) in 31 ps. Although the carboxylate group of Z in principle becomes a stronger base in the excited state, diffusion-controlled protonation would be unable to compete with the decay of Z* at pH values higher than 3 ($1/k_{p1}[H^+]$ > 100 ns). Thus, the net effect of the laser pulse is to perturb the ground-state prototropic equilibrium by producing an excess of A^-. By plotting the observed rate constants (equal to the reciprocal decay times), $k_{obsd} = k_{d2} + k_{p2}[H^+]$, as a function of the proton concentration (Figure 6), the ground-state prototropic rate constants, $k_{d2} = (1.3 \pm 1.1) \times 10^6$ s^{-1} and $k_{p2} = (4.2 \pm 0.1) \times 10^{10}$ M^{-1}·s^{-1}, can be determined. As frequently found for flavylium salts,[4−6] the accuracy of the k_{d2} value determined from the intercept is poor. Alternatively, a more accurate value of $k_{d2} = 6 \times 10^5$ s^{-1} can be calculated from k_{p2} (which is accurate) and the equilibrium constant value of $K_{a2} = 1.5 \times 10^{-5}$. This latter value of k_{d2}, which is within the uncertainty of the intercept value, is the lowest k_d value measured for any of the synthetic or natural flavylium salts studied to date ((1.3−5.0) × 10^6 s^{-1} for HMF, pelargonin, cyanin, DHF, malvin, and oenin).[4−6] This difference suggests that, at best, there is only modest stabilization of the flavylium moiety of Z by the carboxylate charge. In contrast, the value of k_{p2} (4.2 × 10^{10} M^{-1}·s^{-1}) is larger than the k_p values found for the other flavylium salts studied previously ((2.2−3.6) × 10^{10} L·mol^{-1}·s^{-1}), consistent with the fact that the base form of CMHF is negatively charged rather than neutral like the base form of these other flavylium salts.

Excited-State Kinetics. The data from fluorescence decays measured in the pH range from 1.22 to 2.88 (predominant excitation of Z at 445 nm) were analyzed using conventional two-state kinetics for evaluation of the four rate constants in Scheme 3.[1]

The rate constants were evaluated from the two reciprocal decay times (λ_1 and λ_2), the ratio of pre-exponential coefficients at 525 nm ($R = A_{12}/A_{11}$), and the lifetime of Z ($\tau_Z = 1/k_Z$ = 451 ps) measured with the methoxylated parent compound DMF (eqs 1−5).[1]

$$k_{d2}^* = X - k_Z \tag{1}$$

$$k_{p2}^*[H^+] = (XY - \lambda_1\lambda_2)/k_{d2}^* \tag{2}$$

$$k_{A^-} = Y - k_{p2}^*[H^+] \tag{3}$$

$$X = (R\lambda_2 + \lambda_1)/(R + 1) \tag{4}$$

$$Y = \lambda_1 + \lambda_2 - X \tag{5}$$

In the 2−2.88 pH range, $R = \infty$ ($A_{11} = 0$), which implies that $X = \lambda_2$ and $Y = \lambda_1$ (eqs 4 and 5) and that $k_{p2}^*[H^+]$ is vanishingly small (eq 2). Below pH 2, $k_{p2}^*[H^+]$ increases linearly with proton concentration (Figure 7a), with a slope of $k_{p2}^* = (4.4 \pm 0.4) \times 10^9$ M^{-1}·s^{-1}, a value approximately 10-fold smaller than the ground-state k_{p2} (4.2 × 10^{10} M^{-1}·s^{-1}), as expected from the abnormally low values of A_{11}. A simplistic interpretation of this difference between k_{p2}^* and k_{p2} would be a decrease in the base strength of A^- in the excited state, resulting in reaction-controlled protonation in the excited state. However, this is not plausible because such reaction control has not been observed for protonation of any of the other conjugate bases of flavylium photoacids, many of which are even weaker bases. A more consistent interpretation emerges when the pH dependence of k_{A^-} is also considered.

Protonation at the Carboxylate Group. Figure 7b shows plots of the rate constants k_{d2}^* and k_{A^-} versus [H$^+$]. The deprotonation rate constant $k_{d2}^* = 2.9 \times 10^{10}$ s^{-1} is independent of [H$^+$], as expected, and clearly smaller than that of the parent compound DHF ($k_d^* = 8 \times 10^{10}$ s^{-1}).[4]

The linear dependence of k_{A^-} with [H$^+$], with a slope value equal to (3.7 ± 0.8) × 10^{10} M^{-1}·s^{-1} and an intercept value of (8.3 ± 0.08) × 10^9 s^{-1}, is most unusual for two reasons. First, the lifetime of the base form of all other flavylium salts that have been studied is pH independent,[1−3] and second, the lifetime of A^- predicted from the intercept value is 120 ps, clearly shorter than the experimental value (149 ps) measured by direct excitation of A^- at pH 7.2. This suggests that the base form of CHMF is quenched by the proton in a second process, different from back protonation to produce Z*.

Protonation at the carboxylate group, producing the neutral base (AH), is the obvious assignment of this second process because aromatic carboxylate groups become much stronger bases in the excited state (e.g., the naphthoic acids, for which

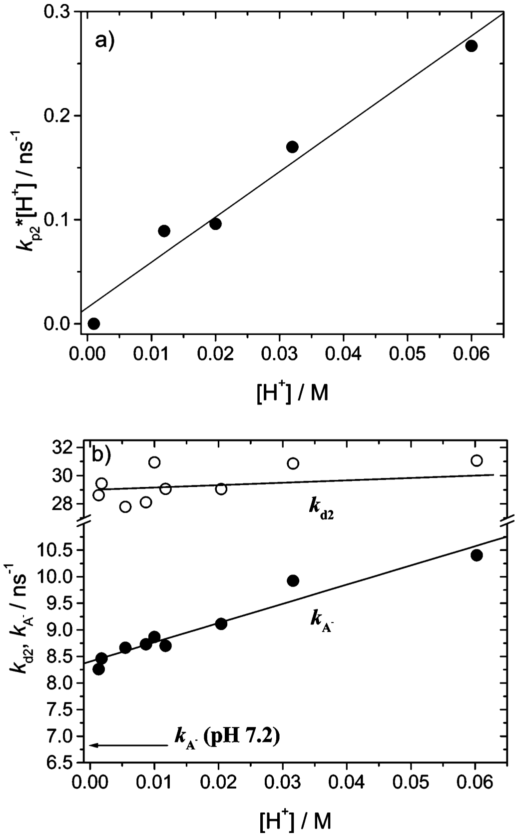


Figure 7. Plots of the rate constants of the excited-state prototropic reactions of CHMF in H_2O as a function of proton concentration: (a) reprotonation rate constant of A^-* at the carbonyl group; (b) deprotonation rate constant of Z* and (apparent) reciprocal decay time of A^-*.

ΔpK_a is ∼6−8 upon excitation)[26] and the carboxylate group bears a negative charge. Protonation at the carboxylate group would not be reflected in the pre-exponential coefficient at 525 nm (A_{11}) because it does not produce Z* and an eventual second protonation on the oxygen at carbon 7 would produce the unstable AH_2^{+}*. In other words, reprotonation at the carboxylate will decrease the apparent lifetime of A^-* without affecting the decay of Z*. In essence, then, the protonation of A^-* at the carboxylate group is in fact diffusion controlled, with a rate constant ($k_{p3}^* = 3.7 \times 10^{10}$ $M^{-1}\cdot s^{-1}$) close to that in the ground state ($k_{p2} = 4.2 \times 10^{10}$ $M^{-1}\cdot s^{-1}$). Scheme 4 summarizes this kinetic situation.

Geminate Recombination. The observed difference ($\Delta k_{A^-} = 1.7 \times 10^9$ s^{-1}) between the value of k_{A^-} measured by direct excitation of A^- at pH 7.2 and that estimated from the intercept of the plot of k_{A^-} versus [H^+] at lower pH values, where A^-* is formed by deprotonation of Z* (see Figure 7b), implies the existence of a pH-independent protonation process of A^-*. This process could be either proton abstraction from water or fast geminate recombination of the A^-*$\cdots H^+$ ion pair formed upon deprotonation of Z*.[27,28] In both cases, the protonation must occur at the carboxylate group (to give AH*), because Z* is not formed from A^-* (single exponential decay). The first hypothesis can be excluded because proton transfer from water is not seen when A^-* is prepared by direct excitation of A^- at pH 7.2. The fact that the geminate pair is an anion−cation pair in the case of CHMF is an additional factor in favor of recombination of the pair due to the reduced rate of proton escape.[28] Thus, we assign the Δk_{A^-} difference to the geminate recombination of the A^-*$\cdots H^+$ pair to give AH*. A more quantitative analysis of the magnitude of Δk_{A^-} is not possible with the present data because it is a combination of three rate constants: proton migration from hydroxyl to carboxylate involving two or three water molecules, dissociation of the ion pair, and recombination of the ion pair. However, it is clear that a difference between the lifetimes of the base form measured by direct excitation or after deprotonation of the acid provides a signature for geminate recombination. In Figure 8, the agreement between the experimental data (decay times and pre-exponential coefficients) and the respective theoretical values derived from the calculated rate constants (Scheme 4 and Table 3) is shown.

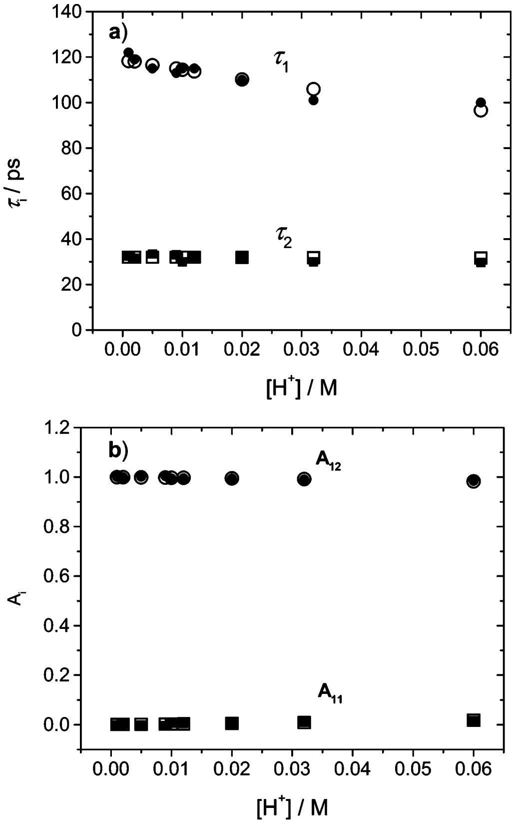


Figure 8. Plots of (a) decay times and (b) pre-exponential coefficients of CHMF in water as a function of proton concentration: experimental (closed symbols) and calculated with the rate constants derived from Scheme 4 (open symbols).

TABLE 3: Proton Transfer Rate Constants for CHMF in Aqueous Solution at 293 K

reaction	rate constant
$AH_2^+ \rightarrow AH$ (ground state) (k_{d1}/s^{-1})	$>7 \times 10^9$
$Z \rightarrow A^-$ (ground state) (k_{d2}/s^{-1})	6.3×10^5
$A^- \rightarrow Z$ (ground state) ($k_{p2}/M^{-1}\cdot s^{-1}$)	4.2×10^{10}
$AH_2^{+*} \rightarrow AH^*$ (excited state) (k_{d1}^*/s^{-1})	1.1×10^{11}
$Z^* \rightarrow A^{-*}$ (excited state) (k_{d2}^*/s^{-1})	2.9×10^{10}
$A^{-*} \rightarrow Z^*$ (excited state) ($k_{p2}^*/M^{-1}\cdot s^{-1}$)	4.4×10^9
$A^{-*} \rightarrow AH^*$ (excited state) ($k_{p3}^*/M^{-1}\cdot s^{-1}$)	3.7×10^{10}

The foregoing results have implications for the analysis and interpretation of the ground-state proton transfer kinetics, because excitation of Z also produces AH (from proton recombination) in addition to A^- (from the natural decay of A^-*). Thus, the return to the Z−A^- equilibrium will also involve deprotonation of AH at the carboxylate followed by protonation on the oxygen at carbon 7, or vice versa, to give Z. However, at the maximum proton concentration used in the light jump experiments (2.3×10^{-2} M), the relative amounts of ground-state A^- and AH produced from A^-* are 80 and 20%, respectively, and part of this AH might undergo reasonably rapid deprotonation to A^-. Thus, the analysis of the ground-state proton transfer rates must be essentially correct.

Conclusions

Nonequilibrated solutions of CHMF exhibit three forms in the ground state between pH 0 and 7: an acid form (AH_2^+), predominant below pH 0.73; a zwitterionic form (Z), predominant in the pH range 0.73−4.84; and an anionic base form (A^-), predominant above pH 4.84. Equilibrated solutions (24 h after preparation) of Z also contain the hemiacetal (B) and the chalcone forms, resulting from hydration of Z (see Scheme 1). The cationic species AH_2^+ is quite a strong acid in the ground state ($k_{d1} > 7 \times 10^9$ s^{-1}) and a super-photoacid in the excited state ($k_{d1}^* \approx 1.1 \times 10^{11}$ s^{-1}). The zwitterion (Z) is a weak acid in the ground state (pK_{a2} = 4.84) but quite strong in the excited state (p$K_{a2}^* < 0$). However, both the ground- and excited-state deprotonation rate constants of Z are significantly smaller than those of the AH^+ form of other flavylium salts, reflecting the presence of the negatively charged carboxylate group in the Z form of CHMF. Although the negative charge is mostly localized on the carboxylate group (the charge on the carboxylate group was evaluated as the sum of the atomic charges on $-CO_2$ calculated at the semiempirical AM1 level), ~40% of the charge is delocalized onto the flavylium ring, increasing the O−H bond strength and causing the deprotonation rate constants to decrease.[4,5] The increased negative charge on the flavylium moiety is also in agreement with the decrease in the extent of hydration observed for the Z form of CHMF relative to the parent compound DHF; any decrease in the net positive charge on carbon 2 will disfavor nucleophilic attack by water.

The most interesting feature of CHMF, however, is the concerted action of the hydroxyl and carboxylate groups upon excitation. Ultrafast photodeprotonation of the hydroxyl group of Z* results in partial geminate recombination with preferential reprotonation at the carboxylate group to give AH*. The net result is proton migration in the excited state between these two positions in the molecule that is fast enough to compete efficiently with dissociation of the geminate pair. The same phenomenon is also reflected in the diffusional reprotonation of A^{-*}, where again the preference is for protonation on the carboxylate group. Thus, the excited Z* form of CHMF effectively acts as a molecular proton transporter in the picosecond time range.

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