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Aust. J. Chem. **2011**, *64*, 23–30

Femtosecond Fluorescence Upconversion Investigations on the Excited-State Photophysics of Curcumin

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The demonstration of curcumin as a photodynamic therapy agent has generated a high level of interest in understanding the photoinduced chemical and physical properties of this naturally occurring, yellow-orange medicinal compound. Important photophysical processes that may be related to photodynamic therapy effects including excited-state intramolecular hydrogen atom transfer (ESIHT) occur within the femtosecond to picosecond time scales. Femtosecond fluorescence upconversion spectroscopy has sufficient time resolution to resolve and investigate these important photophysical processes. In this review, recent advances in using femtosecond fluorescence upconversion to reveal ultrafast solvation and ESIHT of curcumin are presented. The excited-state photophysics of curcumin has been investigated in alcohols and micellar solutions. The results of curcumin in methanol and ethylene glycol reveal the presence of two decay components in the excited-state kinetics with time scales of 12–20 ps and ~100 ps. Similarly, in a micellar solution, biphasic kinetics are present with the fast decay component having a time constant of 3–8 ps, the slow decay component 50–80 ps. Deuteration of curcumin in both media leads to a pronounced isotope effect in the slow decay component, which suggests that ESIHT is an important photophysical process on this time scale. The results of multiwavelength fluorescence upconversion studies show that the fast component in the excited-state kinetics is due to ultrafast solvation. These advances form a part of the continuing efforts to elucidate the photodynamic therapy properties of curcumin.

Manuscript received: 15 November 2010.
Manuscript accepted: 21 December 2010.

Introduction

Curcumin, the yellow pigment in the rhizome of turmeric, has been shown to possess a large number of medicinal effects. Most notably, studies[1,2] have suggested that the low incidence of cancers in the gastrointestinal tract in the population of the Indian subcontinent is related to the intake of curcuminoids, which form a family of yellow polyphenols including curcumin (77%), demethoxycurcumin (17%), bisdemethoxycurcumin (3%), and cyclocurcumin (trace level). In addition to anticancer properties,[3,4] curcumin has anti-inflammatory,[5] antioxidant,[6] anti-Alzheimer's disease,[7] anti-cystic fibrosis,[8] and wound healing effects.[9–11] Recent work shows that curcumin has significant potential as an effective photodynamic therapy agent,[12–19] particularly for melanoma treatment.[20–26] The presence of light has been shown to greatly enhance the destruction of tumour cells.[16,18,19] Although it has been established that stable photoproducts are not the source of medicinal effects of curcumin,[14] photolytically produced reactive oxygen species including singlet oxygen, hydroxyl radical, superoxide, or hydrogen peroxide may be responsible for the photoinduced activity.[13,14,27–31] From the photophysical viewpoint, a study by Jovanovic et al. demonstrates that H-atom transfer is a preferred antioxidant mechanism of curcumin using laser flash photolysis and pulse radiolysis.[32] Currently, there are significant interests in developing a detailed level of understanding of the photophysical and photochemical processes of curcumin in order to further exploit its light-induced medicinal effects.

There are two major challenges in the application of curcumin as an effective treatment agent, namely the lack of bioavailability and the limited stability in aqueous environments. Owing to the low aqueous solubility, curcumin has a tendency to aggregate and precipitate in water, thereby limiting its bioavailability. Moreover, curcumin degrades rapidly in water and buffer solutions with a reaction half-life of 9.5 min at pH 7.2.[33] The degradation involves a retro-aldol condensation reaction catalyzed by the hydroxide anion, to produce products including vanilin, ferulic acid, and feruroyl methane,[34] which have been shown to possess medicinal properties as well. Our studies have shown that encapsulation of curcumin in surfactant micelles and binding to proteins and diamide linked γ-cyclodextrin dimers resolve these two major issues.[35–38]

Micellar systems may play a critical role in enabling the clinical applications of curcumin. These molecular assemblies address the issue of low bioavailability because micelle-captured curcumin is well dispersed in aqueous solutions. Association with the micelle also enables curcumin to be present in a water-free environment,[17,35,37–41] thereby preventing degradation. Investigations on the behaviour of curcumin in micellar systems are crucial because it is largely membrane bound in a biological environment.[42] These studies provide valuable insight into the properties of curcumin in a biologically relevant environment.

Curcumin exists in two tautomeric forms, namely the β-diketone and keto-enol. Payton et al. have shown that

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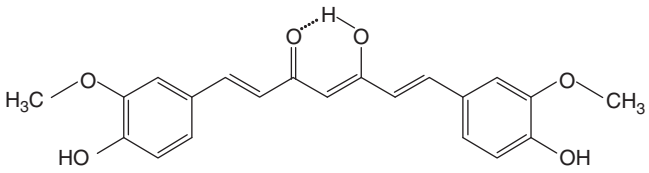

Fig. 1. Structure of keto-enol form of curcumin.

curcumin is predominantly a keto-enol tautomer (Fig. 1) in several solvents with various polarities using results from NMR spectroscopy.[43] This is because a strong intramolecular hydrogen bond is present in the keto-enol tautomer, resulting in stabilization of this tautomeric form. As the keto-enol tautomer, curcumin has the capability to undergo excited state intramolecular hydrogen atom transfer (ESIHT) due to close proximity of the proton donor and the acceptor, as shown in Fig. 1. Our recent work has not only established that ESIHT is a major photophysical event of curcumin, but we have also assigned the time scale of this phenomenon.[44,45] The ESIHT process of curcumin was investigated in two classes of environments. First, studies were performed on curcumin in methanol, ethylene glycol, and chloroform to understand the effect of intermolecular hydrogen bonding on ESIHT.[44] Second, micellar systems composed of sodium dodecyl sulfate (SDS), dodecyl trimethyl ammonium bromide (DTAB), and triton X-100 (TX-100) were chosen to investigate the effect of interfacial water and the charge of the surfactant headgroup on ESIHT.[45] Using deuteration of curcumin and femtosecond fluorescence upconversion spectroscopy, our results show that the time constant of ESIHT of curcumin is 70 ps in methanol and 120 ps in ethylene glycol. In addition, there is a 12–20 ps component in the excited state kinetics that is attributed to ultrafast solvation. In the micellar systems, the results show that the time constant of ESIHT of curcumin in these micelles ranges from 50 to 80 ps. Additionally, a fast component of 3–8 ps, which is due to solvation dynamics of curcumin in the micellar media, was also observed.

It has been suggested that the presence of a labile hydrogen as a result of ESIHT plays a role in the medicinal effects of other naturally occurring pigments such as hypericin and hypocrellin.[46–53] In the case of curcumin, ESIHT may result in partial deprotonation, which induces fragmentation to yield the medicinal degradation products mentioned earlier.

Fluorescence Upconversion Technique

Fluorescence upconversion is one of the most attractive time-resolved fluorescence methods. Compared with similar techniques, it provides the highest time resolution in resolving the decay of fluorescence signals. Typically, the time resolution of fluorescence upconversion is ~300 fs or less. In contrast, although time correlated single photon counting, which is another popular time-resolved fluorescence method, provides a simpler solution to data acquisition, the time resolution is significantly poorer (~50 ps or longer). The apparatus for fluorescence upconversion in our studies is described in detail elsewhere.[54] In short, the laser source was a homebuilt mode-locked Ti:sapphire oscillator. The fundamental wavelength and repetition rate of the femtosecond output were 814 nm and 82 MHz, respectively. The fundamental output of the oscillator was frequency-doubled by a type-I lithium triborate crystal (2 mm). The frequency-doubled pulses (407 nm) were used to excite the sample and the residual of the fundamental was used as the gate pulse to upconvert the fluorescence signal. The polarization of the excitation pulse was at the magic angle relative to that of the gate pulses. First, the frequency-doubled blue pulses (407 nm) were focussed onto a rotating cell containing the sample and the fluorescence signal was collected using a 10× objective lens. Then, the gate pulse and fluorescence signal were focussed onto a 0.4 mm type-I beta barium borate crystal to generate the sum frequency light, which was detected by a photomultiplier tube mounted on a monochromator. The full-width-at-half-maximum of the instrument response function is 300 fs, obtained by the cross-correlation function of the frequency doubled and the fundamental light. All experiments were performed at room temperature. For most fluorescence upconversion experiments, a time window of 100 ps was used with a step size of 0.2 ps.

Solvation Correlation Function

The solvation correlation function, $C(t)$, which reveals the time constants of the solvation process, was used to analyze and quantify the solvation dynamics.

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(\text{`0'}) - \nu(\infty)} \quad (1)$$

The ν(‘0’), $\nu(t)$, and $\nu(\infty)$ in Eqn 1 denote the peak frequency (typically in cm^{-1}) of the zero-time, t and infinity emission spectra, respectively. Using the approach of Fee and Maroncelli,[55] the ‘zero-time’ emission spectrum was approximated by using the emission spectrum of curcumin in hexanes. As for $\nu(\infty)$, the peak frequency of the steady-state fluorescence spectrum was used. In these investigations, fluorescence upconversion measurements were performed at 11–17 wavelengths depending upon the system, ranging from 470 to 630 nm, with a time window of 10 ps to construct time-resolved emission spectra with sufficient data points to reflect the real spectra. Each of the time-resolved emission spectra was fitted with the log-normal function and the peak frequency $\nu(t)$ was obtained using the procedure outlined by Maroncelli and Fleming.[56] The relatively large width of the time dependent emission spectra typically results in uncertainty in the exact position of the maxima. Therefore, by using the signal-to-noise ratio and width of the spectrum (including ‘zero-time’, steady-state, or time-resolved emission spectrum) as sources of uncertainty, we determined the following typical uncertainties: time-resolved emission ($\sim\pm200\ cm^{-1}$), ‘zero-time’, and steady-state ($\sim\pm100\ cm^{-1}$). These uncertainties were used to compute error bars for the $C(t)$. Finally, the fractional solvation at 300 fs was calculated using $f_{300\ fs} = 1 - C(t = 300\ fs)$.

Excited-State Intramolecular Hydrogen Atom Transfer of Curcumin in Methanol, Ethylene Glycol and Chloroform

The excitation and probe wavelengths in the fluorescence upconversion experiments for curcumin in methanol, ethylene glycol, and chloroform are 407 nm and 520 nm, respectively. The excitation wavelength of 407 nm is near the absorption maximum (Accessory Publication), which enables efficient promotion of curcumin from the ground state to the excited state. The fluorescence of curcumin in these solvents at 520 nm is relatively intense (Accessory Publication). Because it is situated on the blue side of the spectrum, the signal reflects early time events in the excited state relaxation process. Fig. 2a shows the fluorescence upconversion signals of curcumin in methanol and deuterated methanol. The signal of curcumin in methanol, which is shown in red, was fitted with a bi-exponential decay function with time constants of 12 ± 2 ps and 70 ± 10 ps with nearly

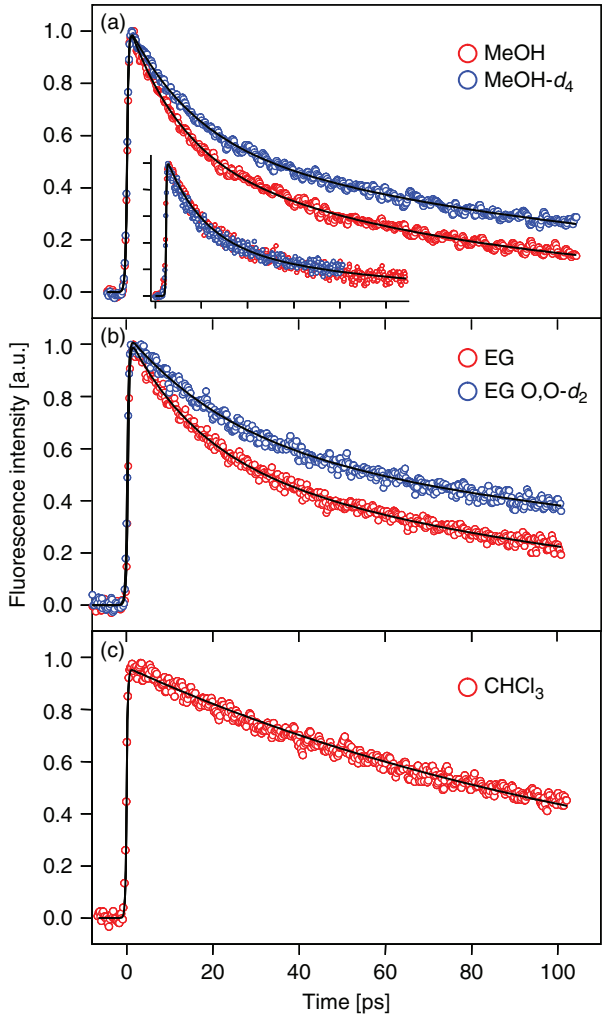

Fig. 2. Fluorescence upconversion decays of curcumin in (a) methanol (MeOH), (b) ethylene glycol (EG) and (c) chloroform ($CHCl_3$); corresponding decays in the deuterated analogs in (a) and (b) show a prominent isotope effect. The inset in (a) shows the virtually identical traces before the H/D exchange (equilibration time of ~15 min), which indicates that the isotope effect observed after equilibration (48 h) is independent of solvent effects. While the decays in (a) and (b) exhibit bi-exponential nature, the trace in (c) is nearly single exponential. All samples were excited at 407 nm and fluorescence was collected at 520 nm. This figure is adapted from ref. [44] and used with permission of the ACS.

equal amplitudes. In deuterated methanol, which is shown in blue, while the fast decay component remains identical within noise level, a significant isotope effect is present in the slow component. As a result, the decay time constant of the slow component is increased to 120 ± 20 ps. The fitting parameters are summarized in Table 1.

Fig. 2b shows the time-resolved fluorescence signal of curcumin in ethylene glycol (red). Similar to methanol, the signal also exhibits a bi-exponential decay. In contrast, the time constants of the decays are longer, which are 20 ± 3 ps and 105 ± 15 ps, with amplitudes of 45% and 55%, respectively. The time-resolved fluorescence of curcumin in deuterated ethylene glycol also produces an isotope effect. While the fast decay component remains unchanged, the time constant of the slow component shows an isotope effect of 2.1. The fluorescence upconversion decay trace of curcumin in chloroform is shown in Fig. 2c, which is best fitted with a single exponential decay function with a time constant of 130 ± 20 ps.

Table 1. Fluorescence upconversion decay parameters for curcumin in different solvents and micellar systems[A]

Solvent/micellar system	a_1	τ_1 [ps]	τ_2 [ps]
Methanol	0.45 ± 0.07	12 ± 2	70 ± 10
Methanol-d_4	0.45 ± 0.07	12 ± 2	120 ± 18
Ethylene glycol	0.45 ± 0.07	20 ± 3	105 ± 15
Ethylene glycol O,O-d_2	0.45 ± 0.07	20 ± 3	220 ± 33
Chloroform[B]	1.0 ± 0.15	130 ± 20	–
TX-100/H_2O	0.30 ± 0.04	8 ± 2	80 ± 10
TX-100/D_2O	0.23 ± 0.03	8 ± 2	130 ± 20
DTAB/H_2O	0.36 ± 0.03	6 ± 2	50 ± 5
DTAB/D_2O	0.22 ± 0.03	6 ± 2	80 ± 5
SDS/H_2O	0.57 ± 0.03	3 ± 2	55 ± 3
SDS/D_2O	0.57 ± 0.03	3 ± 2	85 ± 6

[A]The fluorescence upconversion decays, $f(t)$, were fitted with the multi-exponential function: $f(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2)$, where $a_1 + a_2 = 1$.
[B]Chloroform was dried over molecular sieves before use. Water content was assessed with a coulometric Karl Fischer Titration (Mettler Toledo DL 39). % wt $H_2O = 0.002$.

The emergence of the isotope effect of 1.7 of curcumin in methanol (Fig. 2a) requires equilibration of curcumin in deuterated methanol for ~48 h. It is possible that exchange of the enolic hydrogen of curcumin with the deuterium of methanol-d_4 takes such a long period due to strong intramolecular hydrogen bonding, which is similar to deuteration of a similar system.[57] The inset of Fig. 2a shows the fluorescence upconversion results of curcumin in methanol and deuterated methanol with an equilibration time of only 15 min. In contrast, the two decay traces are identical within experimental error, clearly indicating the absence of an isotope effect. The dependence of the isotope effect on equilibration time offers important insights. First, the absence of any isotope effect with a short equilibration time suggests that the associated excited state process is intramolecular in nature, in contrast to intermolecular processes such as hydrogen bonding interactions between curcumin and the solvent molecules. Second, the observed isotope effect shows a direct correspondence to the enolic H/D exchange of curcumin, supporting the excited state hydrogen atom transfer properties of the kinetics owing to the following reasons. First, it follows that the isotope effect is associated to a non-radiative process as the fluorescence quantum yield of curcumin in methanol has a low value of <10%.[58] Second, it is unlikely that the formation of an excited triplet state by intersystem crossing plays a big role because the triple yield of curcumin is only ~3%.[58] Finally, other non-radiative processes including photoisomerization and ESIHT may also lead to an isotope effect in the excited state kinetics. It is well established that photoisomerization exhibits a strong dependence on solvent viscosity.[59–63] Owing to a weak dependence of the excited state dynamics of curcumin on solvent viscosity, as is discussed below, it is unlikely for photoisomerization to give rise to the observed isotope effect. However, the isotope effect is substantially more likely to arise from ESIHT, which is expected to play a significant role in the deactivation of excited state curcumin due to the presence of a strong intramolecular hydrogen bond in the keto-enol moiety. Because only the slow component in Fig. 2a exhibits an isotope effect, the 70-ps decay component is therefore attributed to ESIHT, which forms a non-radiative decay pathway of curcumin. In the case of curcumin in ethylene glycol, as shown in Fig. 2b, the presence of an isotope effect in the long-lived decay component (105 ± 15 ps) supports the assignment of this

component to ESIHT. The ESIHT time constants of curcumin reported in this study are comparable to that of 7-azaindole in methanol.[64]

The solvent viscosities of methanol and ethylene glycol are 0.59 cP and 16.1 cP, respectively at 20°C. Although the viscosities are different by a factor of ~30 between these solvents, the ESIHT time constants of curcumin in these solvents are relatively similar. The apparent lack of dependence of the ESIHT time constant on viscosity implies that small amplitude molecular motions are involved, which are virtually independent of the surrounding solvent molecules. This phenomenon has been observed in an analogous system, hypericin, of which the ESIHT time constant is unrelated to solvent viscosity.[48,51]

Fluorescence upconversion investigations were also performed on curcumin in chloroform. The results of these studies provide a useful contrast to those of curcumin in methanol and ethylene glycol. Chloroform is a polar aprotic solvent hence unable to form any intermolecular hydrogen bonding with curcumin. In these studies, water was strictly excluded because a trace level of water is able to quench the fluorescence of curcumin.[43] The 130 ± 20-ps decay component is assigned to ESIHT instead of solvation because the time constants of solvation are 0.285 ps and 4.15 ps. This ESIHT time constant is similar to those of curcumin in methanol and ethylene glycol, which implies that intermolecular hydrogen bonding between curcumin and solvent may only play a minor role in ESIHT of curcumin.

Excited-State Intramolecular Hydrogen Atom Transfer of Curcumin in Surfactant Micelles

Fig. 3 presents the femtosecond fluorescence upconversion results of curcumin in the TX-100, DTAB, and SDS micelles. The results of curcumin in both non-deuterated (pH 7.4, 20 mM tris buffer in H_2O, red) and deuterated (pD 7.4, 20 mM tris-d_{11} in D_2O, blue) micellar environments are shown. Similar to curcumin in alcohols, the decay kinetics were monitored at 520 nm with an excitation wavelength of 407 nm. For the SDS micelle, the time-resolved florescence at 520 nm reflects the early time excited-state relaxation dynamics because it is on the blue side of the emission spectrum (Accessory Publication). However, for curcumin in the TX-100 and DTAB micelles, because 520 nm is to the red of the fluorescence maxima, the relaxation dynamics also contain solvation owing to a strong solvatochromatic behaviour of curcumin. The upconversion decays in Fig. 3 are well fitted with a biexponential function within the 100 ps time window and the results are summarized in Table 1. Curcumin in the TX-100, DTAB, and SDS micelles shows a fast component of 8 ± 2 ps, 6 ± 2, and 3 ± 2 ps, respectively, and a slow component of 80 ± 10 ps, 50 ± 5, and 55 ± 3 ps, respectively. Upon deuteration of curcumin in these micelles, the fast component remains unchanged but the slow component experiences a significant isotope effect of 1.6.

The ESIHT time constant of curcumin in the TX-100 micelle, which has a value of 80 ps, is very similar to that of curcumin in methanol (70 ps). It is well accepted that ESIHT of curcumin in methanol is influenced by the interaction between curcumin and the protic solvent molecules.[44,58,65] The agreement in these values indicates the presence of curcumin-water and curcumin-TX-100 intermolecular hydrogen bonding. The presence of these interactions is expected because it has been suggested that curcumin is captured in the palisade layer of the micelle,[58] where a relatively hydrophilic environment is found.[66]

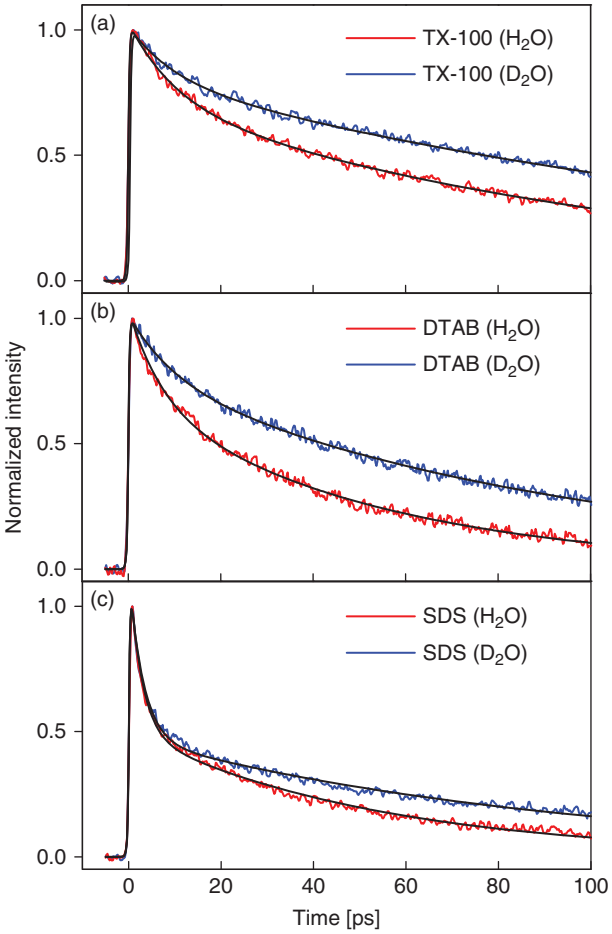

Fig. 3. Fluorescence upconversion decays of curcumin collected at 520 nm in (a) TX-100, (b) DTAB, and (c) SDS micelles at pH = 7.4 tris buffer (red) with an excitation wavelength of 407 nm. Corresponding upconversion decays in deuterated micellar environment at pD = 7.4 (blue) are also included. Curcumin shows a prominent isotope effect in all three micellar media. This figure is adapted from ref. [45] and used with permission of the ACS.

Furthermore, curcumin may form hydrogen bonding interactions with the C–O groups of TX-100, giving rise to an environment that is similar to that of bulk methanol. In the DTAB and SDS micelles, the ESIHT time constants are ~50 ps, which are considerably faster than that in the TX-100 micelle. This faster ESIHT time constant implies a stronger curcumin intramolecular hydrogen bond, and a weaker curcumin-water and/or curcumin-surfactant interaction(s) in these micelles compared with TX-100. First, the water content in the micelles is considered. Results from a study indicate that the water contents of the DTAB and TX-100 micelles are similar but higher in the SDS micelle.[67] Although the DTAB and SDS micelles have different water contents, the nearly identical ESIHT time constants in these micelles imply that the curcumin-water hydrogen bonding only plays an insignificant role in ESIHT. In contrast, the major difference between TX-100 and the other two surfactants is the presence of a high number of C–O groups. As a comparison, DTAB and SDS have the dodecyl group, which is only capable of interacting with curcumin by van der Waals forces. Therefore, it is conceivable that hydrogen bonding between curcumin and TX-100 results in a slower rate of ESIHT, whereas in the DTAB and SDS micelles the ESIHT rate is higher due to the absence of curcumin-surfactant hydrogen bonding.

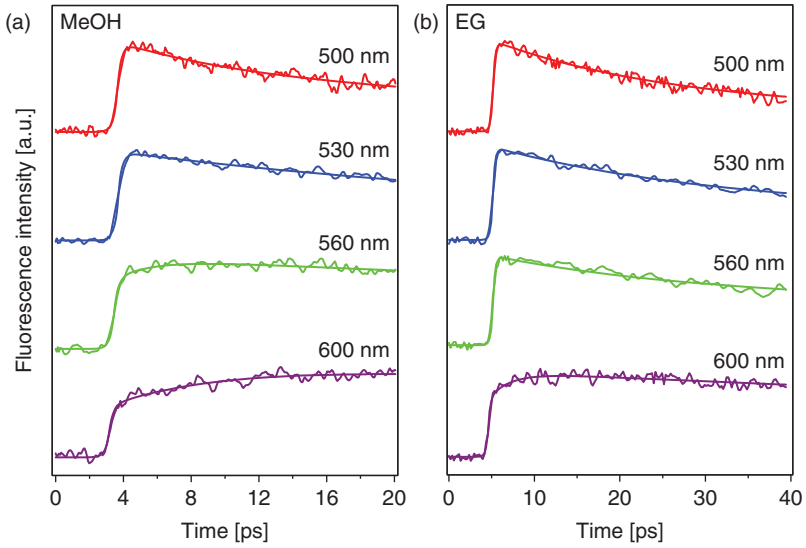

Fig. 4. Representative normalized wavelength resolved fluorescence upconversion traces of curcumin in (a) methanol and (b) ethylene glycol. Time-resolved emission spectra were constructed by collecting upconversion traces over a range from 500 to 600 nm at intervals of 10 nm. The time-resolved traces at the red end (600 nm) show a growing component, which is the signature of solvation dynamics. This figure is adapted from ref. [44] and used with permission of the ACS.

Solvation Dynamics of Curcumin in Methanol and Ethylene Glycol – Ultrafast Solvent Motions

Solvation, the rearrangement of solvent molecules surrounding a dipole, is expected to play a role in the excited state relaxation dynamics of curcumin due to a large dipole moment change of $\Delta\mu = 6.1$ D relative to ground-state curcumin.[58] This magnitude of $\Delta\mu$ is similar to that of Coumarin 153,[56,68] which is commonly used as a standard probe for solvation dynamics. As shown in the Accessory Publication, the fluorescence spectra have a peak shift that is highly sensitive to solvent polarity, which further indicates that solvation plays a major role in the relaxation of the excited state. The presence of solvation can be detected by a multiwavelength fluorescence upconversion investigation. In our study, fluorescence upconversion experiments were performed at up to 11 wavelengths within 500 nm to 600 nm in methanol and ethylene glycol. Several representative decay traces are shown in Fig. 4. The general trend is that on the blue side of the emission spectrum (e.g. 500 nm) the results show a fast and a slow decay component. However, as the wavelength of the fluorescence upconversion experiment increases, the fast decay component gradually transforms into a growth component. The transition from decay to growth in the time-resolved fluorescence signal is due to a decrease in energy of the excited state due to solvation. As mentioned earlier, excited-state curcumin has a significantly higher dipole moment and the excited state in the initial solvent configuration is relatively high in energy. Solvation, which involves rearrangement of the surrounding solvent molecules, takes place in time and it leads to a decrease in the energy of the excited state. The behaviour of the results is an established indication that solvation dynamics are present in the relaxation dynamics of excited state curcumin. A similar behaviour is also observed for curcumin in ethylene glycol.

The wavelength resolved fluorescence upconversion traces are used to construct the time-resolved fluorescence spectra (Accessory Publication) from which the necessary parameters are extracted to generate the solvation correlation function, $C(t)$, which reveals the time constants of solvation dynamics. The $C(t)$ of curcumin in methanol and ethylene glycol are presented in Fig. 5a and the solvation parameters are shown in Table 2. The $f_{300\,\mathrm{fs}}$ values for curcumin in alcohols show that more than 70% of solvation is completed in both the solvents within the time resolution (300 fs) of the instrument. The $C(t)$ of curcumin in methanol and ethylene glycol shows an initial fast response (50 fs, fixed) followed by a slow response; the $C(t)$ for curcumin in these alcohols was fitted with the following multiexponential function and the fitting parameters are listed in Table 2.

$$C(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3 \qquad (2)$$

Previous studies on solvation dynamics of similar systems have shown that the 50-fs component is attributed to ultrafast librational motion of the solvents.[68–70] The slow component in the $C(t)$ has time constants of 12 ± 2 ps and 30 ± 5 ps in methanol and ethylene glycol, respectively. These time constants show excellent agreement with the fast component in the fluorescence upconversion results shown in Fig. 2 and Table 1. Therefore, the fast component in the fluorescence upconversion results for curcumin in methanol and ethylene glycol is assigned to solvation. Furthermore, curcumin in methanol and ethylene glycol have average solvation times of 3.5 ps and 8.2 ps, respectively, showing good agreement with a previous study.[68]

Solvation Dynamics of Curcumin in Surfactant Micelles – Ultrafast Solvation by Interfacial Water

Solvation dynamics in the micellar media is an intense area of research because micelles are realistic models for complex biomembranes. Investigations on solvation dynamics in micelles offer crucial insight into the behaviour of molecules in biomembranes.[71–84] Using the results of curcumin in alcohols as a guide,[44] a series of fluorescence upconversion experiments were conducted with a time window of 10 ps (Fig. 6). For curcumin in the TX-100 micelle (Fig. 6a), the fluorescence upconversion traces show a clear decay at 470 nm (blue) and this decay is lengthened significantly at 540 nm (red). At 610 nm (green), however, the fluorescence decay disappears completely and a rise in fluorescence intensity is observed. As mentioned

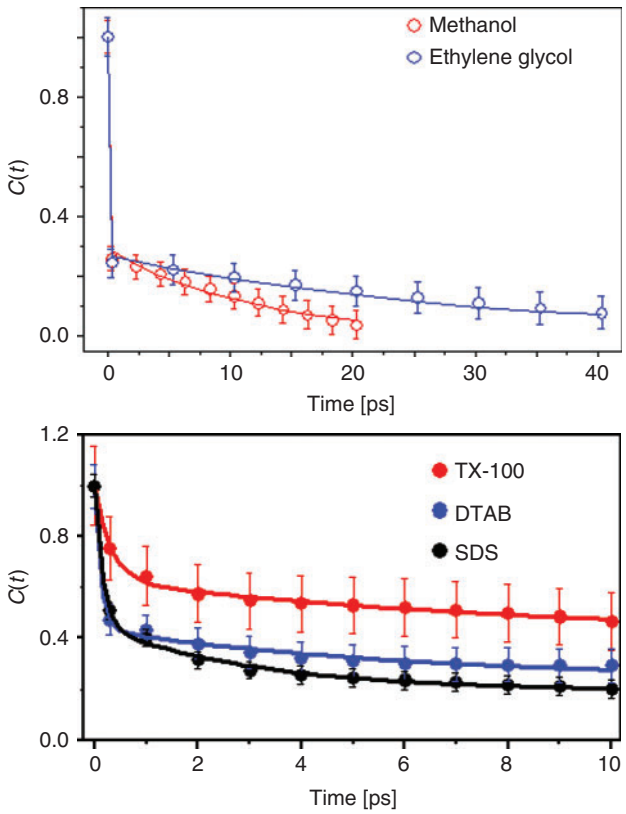

Fig. 5. (Top) The solvation correlation function, $C(t)$ of curcumin in methanol and ethylene glycol obtained from fluorescence upconversion experiments. The $C(t)$ is fitted with a bi-exponential decay function. Both the solvents showed a same initial fast component of 50 fs but a different slow component of 12 ps (methanol) and 30 ps (ethylene glycol). This figure is adapted from ref. [45]. (Bottom) Solvation correlation function, $C(t)$, of curcumin in TX-100 (red), DTAB (blue), and SDS (black) micelles constructed from multiwavelength fluorescence upconversion results. The $C(t)$ is well fitted with a bi-exponential function. All three micelles show different fast and slow components, refer to Table 2. Note that the slow component in $C(t)$ for each micelle is identical to the fast component of the corresponding fluorescence upconversion decay at 520 nm within experimental error. This figure is adapted from ref. [45]. These figures are used with permission of the ACS.

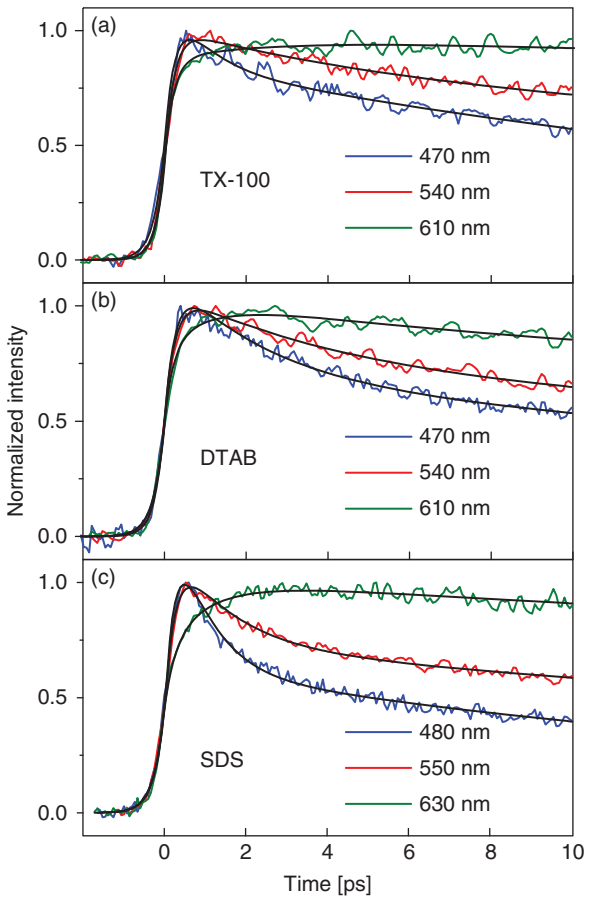

Fig. 6. Representative normalized wavelength resolved fluorescence upconversion decay traces of curcumin at three different wavelengths in (a) TX-100, (b) DTAB, and (c) SDS micelles at pH = 7.4 tris buffer. The time-resolved traces at the red end (610 nm for TX-100 and DTAB and 630 nm for SDS micelles) show a rising component, clearly indicating the presence of solvation dynamics. This figure is adapted from ref. [45] and used with permission of the ACS.

above, the presence of this behaviour is a clear signature of solvation dynamics. For curcumin in the DTAB and SDS micelles (Fig. 6b and 6c), a nearly identical trend in the fluorescence upconversion result is observed. As a whole, the results indicate that solvation dynamics is present for curcumin in TX-100, DTAB, and SDS micelles. Curcumin in the micelles has a $C(t)$ with a rapid decay, a slow decay, and a constant offset (Fig. 5b). The fractional solvation values of curcumin in the TX-100, DTAB, and SDS micelles at 300 fs, ($f_{300\ \mathrm{fs}}$), are 0.25, 0.53, and 0.50, respectively (Table 2). Similar to the cases of curcumin in alcohols, the $C(t)$ for curcumin in each micelle was fitted with Eqn 2. Excellent fits were obtained by fixing the τ_2 component to 8, 6, and 3 ps for the TX-100, DTAB, and SDS micelles, respectively. In our study, the τ_2 value of $C(t)$ for each micelle is virtually identical within experimental error to that of the fast component of the corresponding fluorescence upconversion results (Fig. 3 and Table 1). The agreement indicates that the fast component in the fluorescence upconversion results is due to solvation dynamics of curcumin in the micellar media. The time constants of the fast component in the $C(t)$ are 0.31 ps, 0.12 ps, and 0.15 ps for curcumin in the TX-100, DTAB, and SDS micelles. These time constants are close to or less than the instrument response function (IRF) of our apparatus, with an amplitude of 37%, 56%, and 54%, respectively. This rapid decay of $C(t)$ is followed by a slow decay component, of which the time constants are fixed as mentioned above. The amplitudes of this component are nearly the same in the three micelles, ranging from 20 to 26%. The results in Fig. 5b also show that solvation dynamics are incomplete within the detection time window, resulting in a constant offset in the $C(t)$, which is represented as the a_3 component in Eqn 2.

The decay of $C(t)$ of curcumin in the TX-100, DTAB, and SDS micelles show a fast component with a time constant that is equal or faster than the IRF (300 fs) our apparatus. For the DTAB and SDS micelles, this fast solvation dynamics has been previously observed and attributed to motions of labile or bulk-like water molecules at the interface of the micelle.[71–74,84] However, for TX-100 micelle, the fastest component observed before our work has a time constant of 2.1 ps,[81] which is longer than the measured value of ~300 fs in our study. This ~300 fs solvation dynamics is previously unobserved, and our result is the first demonstration of ultrafast solvation dynamics of the TX-100 micelle. The fastest component in the $C(t)$ is a likely result of interaction of curcumin with the bulk-like water molecules at the aqueous/micelle interface. A previous study shows that the hydration layer of the SDS and DTAB-like

Table 2. Solvation correlation function, $C(t)$, decay parameters for curcumin in different systems

	$f_{300\,fs}$[A]	a_1[B]	τ_1[C] [ps]	a_2	τ_2 [ps][D]	a_3	$\langle\tau\rangle$ [ps]
Methanol	0.75 ± 0.04	0.71 ± 0.02	0.05	0.29 ± 0.02	12 ± 2	–	3.5
Ethylene glycol	0.75 ± 0.05	0.73 ± 0.02	0.05	0.27 ± 0.02	30 ± 5	–	8.2
TX-100	0.25 ± 0.03	0.37 ± 0.02	0.31 ± 0.04	0.22 ± 0.03	8	0.41 ± 0.02	1.9
DTAB	0.53 ± 0.03	0.56 ± 0.02	0.12 ± 0.02	0.20 ± 0.02	6	0.24 ± 0.02	1.3
SDS	0.50 ± 0.03	0.54 ± 0.02	0.15 ± 0.02	0.26 ± 0.02	3	0.20 ± 0.01	0.9

[A]$f_{300\,fs}$: fractional solvation at 300 fs.
[B]The $C(t)$ was fitted with the multiexponential function $C(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3$.
[C]The faster component was fixed at 0.05 ps during fitting.
[D]The τ_2 component was fixed at 8, 6, and 3 ps for the TX-100, DTAB, and SDS micelles, respectively to demonstrate the agreement with the results in Fig. 3 and Table 1.

micelles is considerably thinner (6–9 Å) than that of the TX-100 micelle (20 Å),[72] indicating that curcumin is more exposed to the bulk-like water molecules in the former two micelles than in the latter. As a result, faster solvation dynamics are observed in the SDS and DTAB micelles than TX-100. The 8, 6, and 3-ps component of $C(t)$ in the TX-100, DTAB, and SDS micelles, respectively, shows good agreement with previous results, which has been attributed to solvation of water molecules that are bound to the surface of the micelle by hydrogen bonding.[75,81] The presence of a constant offset in the $C(t)$ of curcumin in the micelles, a_3 (Table 2), indicates that there is a solvation dynamics component that is substantially longer than the time window (10 ps) of the studies. It has been established in previous studies that $C(t)$ has a long lived component in micelles, with time constants ranging from 165 to 300 ps.[75,81]

Conclusion

We have demonstrated with fluorescence upconversion that curcumin undergoes ESIHT and it plays a major role in the photophysics of curcumin. Photoexcitation of curcumin in methanol and ethylene glycol produces a fluorescence signal which decays with a bi-exponential fashion on the order of 12–20 ps and 70–105 ps. The long-lived signal, which exhibits a prominent isotope effect in deuterated solvents, is attributed to ESIHT. We have also shown that curcumin exhibits a long solvation component of ~12 ps in methanol and ~30 ps in ethylene glycol. In addition to alcohols, ESIHT is also a major photophysical process of curcumin in the TX-100, DTAB, and SDS micelles. The fluorescence upconversion transient shows a bi-exponential decay, with time constants of 3–8 ps (fast) and 50–80 ps (slow). Similar to the cases of curcumin in alcohols, the slow component exhibits a pronounced isotope effect, producing a decay time constant of 80–130 ps, which is assigned to ESIHT. The ESIHT rate of curcumin in the TX-100 micelle is lower than those in the other two micellar system because the hydrogen bonding between curcumin and the TX-100 surfactant may contribute to this effect. The fast decay component, however, is insensitive to deuteration of curcumin and has been attributed to solvation dynamics. The water molecules at the micelle interface, which are labile or bulk-like, and those that are bound to the surface of the micelle give rise to solvation dynamics of curcumin. Our work in this area forms a part of the continuing efforts to elucidate the photodynamic therapy properties of curcumin.

Accessory Publication

Supplementary UV-Vis, steady-state and time-resolved fluorescence spectra are available on the Journal's website.

Acknowledgement

T.W.K. acknowledges a research grant from the Australian Research Council and National Health and Medical Research Council Network 'Fluorescence Applications in Biotechnology and Life Sciences'. We dedicate this review to the late Professor Paul F. Barbara for his pioneering work in the field of proton transfer in liquids.

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