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

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Femtosecond fluorescence upconversion experiments were performed on the naturally occurring medicinal pigment, curcumin, in anionic, cationic, and neutral micelles. In our studies, the micelles are composed of sodium dodecyl sulfate (SDS), dodecyl trimethyl ammonium bromide (DTAB), and triton X-100 (TX-100). We demonstrate that the excited-state kinetics of curcumin in micelles have a fast (3–8 ps) and slow (50–80 ps) component. While deuteration of curcumin has a negligible effect on the fast component, the slow component exhibits a pronounced isotope effect of ~1.6, indicating that micelle-captured curcumin undergoes excited-state intramolecular hydrogen atom transfer. Studies of solvation dynamics of curcumin in a 10 ps time window reveal a fast component (<300 fs) followed by a 8, 6, and 3 ps component in the solvation correlation function for the TX-100, DTAB, and SDS micelles, respectively.

Introduction

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, has received considerable attention owing to its numerous medicinal properties. Curcumin, whose structure is shown in Figure 1, is the major ingredient of the yellow pigments (curcuminoids) in the Indian spice plant turmeric. While curcuminoids are composed of approximately 77% curcumin, demethoxy curcumin (17%) and bisdemethoxy curcumin (3%) constitute most of the remaining portion. In addition, cyclocurcumin, which was isolated in 1993, is also present at a trace level. A large number of studies have shown that curcumin possesses anticancer, anti-Alzheimer, anticytisic fibrosis, and other desirable medicinal benefits. Of particular interest are the prospects of curcumin or curcumin-like nontoxic agents to exhibit anticancer effects without side effects, unlike conventional chemotherapy drugs. Several clinical trials are either underway or have recently been completed with curcumin as the treatment agent. The preliminary results from these trials are highly promising.

Two major challenges in the application of curcumin as an effective treatment agent are the lack of bioavailability and severely limited stability in aqueous environments. Because of the low aqueous solubility, curcumin tends to aggregate and precipitate in water, limiting its bioavailability. In addition, curcumin undergoes rapid degradation in water and buffer solutions, with a reaction half-life of 9.5 min at pH 7.2. It has been demonstrated that the degradation is mostly due to deprotonation of curcumin, producing the degradation products vanilin, ferulic acid, and feruroyl methane. It is interesting that most of these products have medicinal properties as well. Previous studies have shown that encapsulation of curcumin in surfactant micelles and binding to proteins resolve these two major issues.

Micellar systems may potentially play an important role in leading to improved clinical use of curcumin. It is established that micelle-captured curcumin is well-dispersed in aqueous solutions, thereby increasing the bioavailability significantly. Moreover, curcumin is trapped in the regions of the micelle where the presence of free water molecules is relatively limited, preventing alkaline hydrolysis, which is the major mechanism for degradation. It is therefore plausible that association to micelles plays a considerable role in enabling curcumin to exhibit its medicinal characteristics. Apart from preventing degradation, micelles also serve as well-defined model systems for biomembranes. It has been shown that a large portion of curcumin is membrane-bound in a biological environment. Investigations on the behavior of curcumin in micelles may provide valuable insight into the properties of curcumin in biomembranes.

Curcumin exists predominantly as the keto–enol tautomter in a number of solvents. It is capable of executing excited-state intramolecular hydrogen atom transfer (ESIHT) due to the presence of strong intramolecular hydrogen bonding between the proton donor and the acceptor atom, as indicated in Figure 1. Although there is a general agreement that ESIHT is a major photophysical event of curcumin, the exact time scale of this phenomenon was not well-established until our recent work. Using deuteration of curcumin and time-resolved fluorescence upconversion as a technique for probing ESIHT, our work has shown that the time constant of ESIHT of curcumin is 70 ps in methanol and 120 ps in ethylene glycol. It has been proposed 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. As for curcumin, it is possible that ESIHT results in partial deprotonation, which induces fragmentation to yield the medicinal degradation products mentioned earlier. While ESIHT of curcumin in polar organic solvents has been established, it remains unclear if micelle-captured curcumin also undergoes this photoinduced process. It is known that intramo-
molecular proton-transfer processes are affected in confined environment due to the structure, various interfaces, and dynamics of the environment.32

Here, we present unambiguous results demonstrating that ESIHT is a major photophysical event of curcumin in micelles. The well-studied micellar systems composed of sodium dodecyl sulfate (SDS), dodecyl trimethyl ammonium bromide (DTAB), and triton X-100 (TX-100), which are anionic, cationic, and nonionic in nature, respectively, were chosen for our investigations. These micelles not only serve as model systems for biomembranes but also enable photophysical investigations as a function of the polarity of the head group. By comparing the excited-state decay kinetics of the deuterated and nondeuterated species obtained by fluorescence upconversion spectroscopy, we show that the time constant of ESIHT of curcumin in these micelles ranges from 50 to 80 ps. In addition to ESIHT, a fast component of 3–8 ps was also observed. Results from multiwavelength fluorescence upconversion studies reveal that the fast component is due to solvation dynamics of curcumin in the micellar media. In short, the present studies offer the first insights into the photophysical events of curcumin in micelles, which may lead to further understanding of the behavior of curcumin in biomembranes.

Experimental Section

Materials. Curcumin (purity ⩾ 98.5%) were purchased from Sigma Aldrich and Alexis Biochemicals, respectively. High-purity curcumin was used for all experiments except in the solvation dynamics studies. We confirmed that curcumin from the two sources produces identical fluorescence upconversion results at 520 nm. Triton X-100 (TX-100, reduced), dodecyl trimethyl ammonium bromide (DTAB, ⩾99%), and sodium dodecyl sulfate (SDS, ⩾99%) were obtained from Sigma Aldrich and used without further purification. Tris-<sup>d<sub>4</sub></sup> solution (1 M in D<sub>2</sub>O, 98 atom % D), deuterium chloride (35 wt % solution in D<sub>2</sub>O, 99 atom % D), and D<sub>2</sub>O (99.9 atom % D) were purchased from Sigma Aldrich. The nondeuterated tris (>99.8% purity) was acquired from AMRESCO. Methanol and methanol-<sup>d<sub>4</sub></sup> (purity 99.8%) were obtained from Fisher Scientific and Cambridge Isotope Laboratories, Inc., respectively, and used as received. All solutions were prepared with water from a Millipore Milli-Q NANOpure water system.

Sample Preparation. A 20 mM buffer solution was prepared at either pH or pD = 7.4 for all of the experimental work in these studies using tris or tris-<sup>d<sub>4</sub></sup>, respectively. The concentration of surfactant was maintained at 0.1 M, which is above the CMC for each of the micelles, in nondeuterated and deuterated tris buffer. Two stock solutions of curcumin in methanol and methanol-<sup>d<sub>4</sub></sup> were used for steady-state, time-correlated single-photon counting (TCSPC) measurements. A small quantity (2 µL) of the curcumin stock solution (4.5 or 1.5 mM) was transferred to the micellar solution to yield a solution with a curcumin concentration of 3 or 1 µM for the UV-vis or fluorescence spectra, respectively. Care was taken to keep the total methanol content of the final sample solution at <0.1% for all solutions. The solutions were then allowed to equilibrate overnight in either the tris/H<sub>2</sub>O or tris-<sup>d<sub>4</sub></sup>/D<sub>2</sub>O solution in the dark prior to the optical experiments. Equilibration was particularly important for curcumin in the D<sub>2</sub>O micellar solutions to ensure a complete exchange of the enolic hydrogen of curcumin with deuterium, as indicated below. For the upconversion measurements, the required amount of curcumin (powder form) was directly added to the micellar solution to result in a curcumin concentration of 0.5–1 × 10<sup>−3</sup> M. This solution was then sonicated for 1 h in the dark. All of the solutions for fluorescence upconversion measurements were allowed to equilibrate overnight. In addition, data collected with the deuterated samples that were equilibrated for 48 h are identical to those with only overnight equilibration, indicating completion of H/D exchange.

Steady-State Measurements. Steady-state UV–vis absorption and emission spectra were acquired on a Hewlett-Packard 8453 UV–visible spectrophotometer and a Spex Fluoromax-4 with 1 nm resolution at room temperature. The emission spectra were corrected for lamp spectral intensity and detector response. The emission spectra were obtained with an excitation wavelength of 407 nm with a 3 nm band-pass for all of the samples. A 5 mm path length quartz cuvette was used for all of the absorption and emission measurements.

Time-Resolved Measurements. Excited-state lifetime measurements were performed using the TCSPC technique. A homebuilt mode-locked Ti:sapphire oscillator pumped by a Nd:VO<sub>4</sub> laser (Millennia, Spectra Physics) producing femtosecond pulses tunable from 780 to 900 nm with a repetition rate of 82 MHz was used as the laser source. The fundamental wavelength at 814 nm from the Ti:sapphire oscillator was modulated by a Pockels cell (Model 350-160, Conoptics Inc.) to reduce the repetition rate to approximately 8.8 MHz and was subsequently frequency-doubled by using a harmonic generator (Model TP-2000B, U-Opal Technologies). The resulting blue light, which had a central wavelength of 407 nm, provided the excitation source. The fluorescence was collected at a 90° geometry and passed through an analyzer set at the magic angle (54.7°) with respect to excitation polarization. A half-wave plate before a vertical polarizer ensured the polarization of the excitation light. A 425 nm cutoff filter was placed in front of a multichannel plate, MCP (Hamamatsu). The detector output was amplified and fed to the Becker & Hickl photon counting module Model SPC-630. The full width at half-maximum (fwhm) of the instrument response function was ∼40–45 ps. All of the measurements were made in a 3.33 ns time window with a total of 1024 channels. A total of 65530 counts were collected at the peak channel for all of the lifetime measurements. A cuvette of 1 cm path length was used for all of the lifetime measurements.

The apparatus for fluorescence upconversion is described in detail elsewhere.33 In short, the laser source was also a home-built 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 from the oscillator was frequency-doubled by a type-I LBO 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 focused 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 focused onto a 0.4 mm type-I BBO crystal to generate the sum frequency light, which was detected by a photomultiplier tube mounted on a monochromator. The full width at half-maximum (fwhm) of the instrument response function was 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.
The solvation correlation function, \( C(t) \), was used to analyze and quantify the solvation dynamics

\[
C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)}
\]  

(1)

The \( \nu(0) \), \( \nu(t) \), and \( \nu(\infty) \) in eq 1 denote the peak frequency (typically in \( \text{cm}^{-1} \)) of the zero time, \( t \), and infinity emission spectra. Using the approach of Fee and Maroncelli,\textsuperscript{34} 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 15–17 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.\textsuperscript{35} 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 (\( \sim \pm 200 \text{ cm}^{-1} \)) and zero time and steady-state (\( \sim \pm 100 \text{ cm}^{-1} \)). These uncertainties were used to compute error bars for the \( C(t) \). Finally, the fractional solvation at 300 fs was calculated using \( f_{300 \text{fs}} = 1 - C(t=300 \text{ fs}) \).

Results and Discussions

**UV–Vis Absorption and Emission Spectra of Curcumin in Micelles.** The spectral properties of curcumin in three different micelles were investigated systematically to examine the influence of micellar environments on spectral features. Figure 2 depicts the UV–vis absorption and emission spectra of curcumin in TX-100, DTAB, and SDS micelles under deuterated (blue) and nondeuterated (red) conditions at pH 7.4 in tris buffer. Curcumin exhibits broad and intense absorption peaks at 426, 423, and 432 nm in the TX-100, DTAB, and SDS micelles, respectively. A small shoulder in the absorption band is present at 445 and 442 nm in the TX-100 and DTAB micelles, whereas curcumin produces a featureless absorption band in the SDS micelle. The absence of vibronic structure in the absorption band of curcumin, as observed in the SDS micelle, is also observed for curcumin in water. This phenomenon indicates that curcumin may interact strongly with water molecules in the Stern layer of the micelle, which is the layer between the core/water interface and the hydrodynamic shear surface,\textsuperscript{36} as will be discussed in more detail forthwith. Additionally, a broad spectral shoulder is present at around 500 nm for curcumin in the DTAB micelle. This extra shoulder, which is absent in the other two micelles, is due to the presence of a small population of deprotonated curcumin at pH = 7.4.

As for the emission spectrum, curcumin shows a broad spectrum peaked at 501, 498, and 548 nm in the TX-100, DTAB, and SDS micelles, respectively. The peak positions of the UV–vis absorption and emission spectra of curcumin are affected by the isotopic substitution, as shown in Figure 2. While a 3 nm blue shift appears in the UV–vis absorption of curcumin in both the TX-100 and DTAB micelles in D\(_2\)O, a red shift with the same magnitude in the emission maximum emerges in these micelles. As for the SDS micelle in D\(_2\)O, a 3 nm blue and \( \sim \)10 nm red shift are found in the absorption and emission maxima, respectively. The changes in absorption and emission peak positions are possibly due to changes in zero-point energies in \( S_0 \) and \( S_1 \) induced by deuteration.\textsuperscript{37,38}

A comparison between the UV–vis absorption and emission spectra of curcumin in micelles with those in a number of organic solvents and water provides valuable insights. The UV–vis absorption spectra of curcumin in the TX-100 and DTAB micelles resemble those of curcumin in aprotic solvents, including chloroform and toluene,\textsuperscript{23,39} suggesting similarities between the environments. However, the emission spectra of curcumin in these micelles are red-shifted compared to those in the aprotic solvents, potentially due to interactions with water molecules at the interface and within the micelle,\textsuperscript{40–44} as will be discussed further in a later section. It is well established that curcumin exhibits a red-shifted emission spectrum in protic solvents including methanol and water, with an emission maximum at around 550 nm. The overall results suggest that curcumin is likely to be located in the palisade layer of the TX-100 and DTAB micelles, which is consistent with results from another study.\textsuperscript{22} As for the SDS micelle, the positions of the UV–vis absorption and emission maxima are identical to those of curcumin in methanol and water, indicating that the micellar environment is analogous to the polar environment in these protic solvents. This suggests that curcumin is probably situated near the Stern layer of the SDS micelle, which facilitates strong interactions with bulk water.

The Stokes shifts of curcumin are 75, 75, and 116 nm in the TX-100, DTAB, and SDS micelles, respectively. Interestingly, the values of the Stokes shift for TX-100 and DTAB micelles are similar to those measured for human and bovine serum albumin (HSA and BSA) bound curcumin,\textsuperscript{14,45–48} It is established that curcumin binds strongly in the hydrophobic pocket of these proteins. The similar Stokes shift values for TX-100 and DTAB micelles imply that the micellar environment is

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**Figure 2.** UV–vis absorption and emission spectra of curcumin in (A) TX-100, (B) DTAB, and (C) SDS in H\(_2\)O (red) and D\(_2\)O (blue).
H2O system shows a fast component of 80 ps in TX-100. In DTAB/D2O and SDS/D2O media, respectively. Deuteration of curcumin induces a similar isotope effect, that is, an identical fast component and lengthening of the slow component. The time constants of the long component are 80 ± 5 and 85 ± 6 ps in the DTAB/D2O and SDS/D2O media, respectively. The presence of an isotope effect of ~1.6 for curcumin in these two micelles reinforces our assignment of the long-lived component to ESIHT. As for the fast component observed in the results, further discussion will be given in the following section.

Several studies have shown that the ESIHT process in curcumin is highly sensitive to the presence of hydrogen bonding between curcumin and the solvent.21–23 Nardo et al. and Khopde et al. independently reported that there is a ~50 ps decay component in the fluorescence lifetime (89 and 64 ps, as measured by the two groups, respectively) of curcumin in cyclohexane using TCSPC.21,22 This decay component is, however, limited by the time resolution of the TCSPC apparatus. It is accepted that this fast decay component is due to ESIHT of curcumin.22 The ESIHT time constant of curcumin in this nonpolar solvent is shorter than that in methanol, which has a value of ~70 ps.23 The results imply that the strength of the intramolecular hydrogen bond of curcumin is the determining factor in the rate of ESIHT. These authors, furthermore, suggest that in protic solvents, such as methanol and ethanol, the keto–enol group of curcumin forms strong hydrogen bonds with the solvent molecules, in addition to the intramolecular hydrogen bond within the keto–enol group.21,22 The interactions between curcumin and the protic solvent molecules interfere with the intramolecular hydrogen bond, weakening the bond effectively, thereby decreasing the rate of ESIHT. However, such perturbation in intramolecular hydrogen bonding is absent in nonpolar solvents such as cyclohexane, enabling a strong intramolecular hydrogen bond in the keto–enol moiety of curcumin and facilitating a fast ESIHT.

The ESIHT time constant of curcumin in the TX-100 micelle, with a value of 80 ps as measured in this fluorescence upconversion study, is very similar to that of curcumin in methanol (70 ps), as reported in our previous study using the same technique.25 Additionally, the ESIHT time constant of curcumin reported in this study is comparable to that of 7-azaindole in methanol.49 The good agreement indicates a substantial level of curcumin–solvent and curcumin–surfactant intermolecular hydrogen bonding in the micelle. This result is consistent with a previous study, suggesting that curcumin is trapped in the palisade layer of the micelle,22 which is a relatively hydrophilic layer of the micelle.50 In addition, studies have shown that the C–O groups in TX-100 interact favorably with water, which supports that there is a substantial level of

Fluorescence Upconversion and Excited-State Intramolecular Hydrogen Atom Transfer of Micelle-Captured Curcumin. The fluorescence upconversion results of curcumin in the TX-100, DTAB, and SDS micelles are shown in Figure 3. The fluorescence upconversion decay traces of curcumin in both nondeuterated and deuterated micellar environments at pH and pD = 7.4, respectively, are illustrated in the figure. The decay kinetics were monitored at 520 nm with an excitation wavelength of 407 nm. Collection of time-resolved fluorescence at 520 nm in (A) TX-100, (B) DTAB, and (C) SDS micelles at pH 7.4 (blue) are also included. Curcumin shows a prominent isotope effect in all three micellar media.

Similar to the hydrophobic regions of HSA and BSA. In contrast, the sizable Stokes shift observed in the SDS micelle is virtually identical to that observed in a buffer solution, which reflects that the local environment of curcumin in the SDS micelle may have a significant bulk water content, which further supports that curcumin is located near the Stern layer of the micelle.

Table 1: Fluorescence Upconversion Decay Parameters for Curcumin in Different Micellar Systems

<table>
<thead>
<tr>
<th>micellar system</th>
<th>(a_1) (±)</th>
<th>(\tau_1) (ps)</th>
<th>(\tau_2) (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX-100/H2O</td>
<td>0.30 ± 0.04</td>
<td>8 ± 2</td>
<td>80 ± 10</td>
</tr>
<tr>
<td>TX-100/D2O</td>
<td>0.23 ± 0.03</td>
<td>8 ± 2</td>
<td>130 ± 20</td>
</tr>
<tr>
<td>DTAB/H2O</td>
<td>0.36 ± 0.03</td>
<td>6 ± 2</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>DTAB/D2O</td>
<td>0.22 ± 0.03</td>
<td>6 ± 2</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>SDS/H2O</td>
<td>0.57 ± 0.03</td>
<td>3 ± 2</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>SDS/D2O</td>
<td>0.57 ± 0.03</td>
<td>3 ± 2</td>
<td>85 ± 6</td>
</tr>
</tbody>
</table>

\(*\) The fluorescence upconversion traces, \(f(t)\), were fitted with the multiexponential function \(f(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2)\), where \(a_1 + a_2 = 1\).
water in the micelle.\textsuperscript{51,52} Furthermore, curcumin may also have considerable hydrogen bonding with oxygen in the C–O groups in the TX-100 surfactants, as will be discussed below. In short, in the case of the TX-100 micelle, our results indicate that hydrogen bonding between curcumin and the micellar environment is similar to that between curcumin and bulk methanol, which is consistent with results from a previous report.\textsuperscript{22}

In the DTAB and SDS micelles, the ESIHT time constants are approximately 50 ps, as shown in Table 1. It appears that the fast ESIHT times observed in these micelles compare well with those in the nonpolar solvent cyclohexane.\textsuperscript{51,22} However, as indicated earlier, the ESIHT process of curcumin in cyclohexane is most likely faster than what was measured with TCSPC due to limited temporal resolution. In comparison to curcumin in the TX-100 micelle, the ESIHT process occurs faster in the DTAB and SDS micelles. It is obvious that there is a stronger curcumin intramolecular hydrogen bond and a weaker curcumin–water and/or curcumin–surfactant interaction in these micelles compared to those in TX-100. To gain insight into the higher rates of ESIHT in the DTAB and SDS micelles relative to that of TX-100, we first consider the water content of the micelles. Studies have suggested that the water contents are similar for the DTAB and TX-100 micelles but higher in the SDS micelle.\textsuperscript{53} Therefore, it follows that the curcumin–water hydrogen bonding is likely to play a minor role in leading to a higher rate of ESIHT for curcumin in the DTAB and SDS micelles. An obvious difference between TX-100 and the other two surfactants is the presence of a high number of C–O groups in the backbone of TX-100, which may interact with curcumin through hydrogen bonding. In contrast, the surfactant backbones of DTAB and SDS are composed of the dodecyl group, which only interacts with curcumin through weak van der Waals forces. It is likely that the presence of a considerable hydrogen bonding network for curcumin in the TX-100 micelle leads to a slower rate of ESIHT. In the DTAB and SDS micelles, however, the ESIHT rate is higher due to the absence of such a hydrogen bonding network. While the results in this study are consistent with the notion of additional hydrogen bonding in the TX-100 micelles for curcumin, more work will be necessary to understand this effect fully.

Early Time Solvation Dynamics in Micelles. The fluorescence upconversion results in Figure 3 show that in addition to the component that is attributed to ESIHT, there is an early time decay component with a time constant significantly shorter than the ESIHT component. Table 1 summarizes the time constant of the fast component, showing that values of 8, 6, and 3 ps were obtained for curcumin in TX-100, DTAB, and SDS micelles, respectively. Interestingly, unlike the ESIHT component which shows an isotope effect, the fast decay component is insensitive to deuteration of curcumin. As shown in Table 1, the \( t_1 \) values are virtually identical for nondeuterated and deuterated curcumin in each of the micelles. In our previous study of curcumin in polar organic solvents, an isotope-effect-free fast decay component was also observed, and we demonstrated that this decay component is due to solvation of curcumin in these solvents.\textsuperscript{23} Using the previous results as a guide, we conducted a series of fluorescence upconversion experiments with a time window of 10 ps. The results are shown in Figure 4. For curcumin in the TX-100 micelle (Figure 4A), the fluorescence upconversion traces show a clear decay at 470 nm (blue), and this decay slows down considerably at 540 nm (red). However, at 610 nm (green), the fluorescence decay vanishes, and a rise component is observed. The presence of the decay and rise components as a function of fluorescence wavelength shown in Figure 4 is a key signature of solvation dynamics. As will be discussed further below, water molecules in the micellar structure play a major role in solvating curcumin. For curcumin in the DTAB micelle (Figure 4B), an almost identical trend in the fluorescence upconversion is shown, with a rise component observed at 610 nm. While a similar behavior of the multi-wavelength fluorescence upconversion is shown by curcumin in the SDS micelle, the wavelengths at which the decays were recorded were set slightly to the red due to a larger Stokes shift observed for curcumin in this micelle. In short, the results indicate the presence of solvation dynamics for curcumin in TX-100, DTAB, and SDS micelles.

Using the method outlined in the Experimental Section, the solvation correlation functions, \( C(t) \), for curcumin in the three micelles were constructed from 15–17 wavelength-resolved decay traces depending on the systems and are shown in Figure 5. First, it is clear that for curcumin in each micelle, \( C(t) \) shows a rapid decay which is followed by a slow decay and an apparent constant offset. Curcumin shows a fractional solvation at 300 fs, \( f_{300} \), of 0.25, 0.53, and 0.50, in the TX-100, DTAB, and SDS micelles, as summarized in Table 2. The \( C(t) \) for curcumin in each of the micelles was fitted with a multieponential function, of which the equation is shown as follows, and the fitting parameters are listed in Table 2.

\[
C(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3
\]  

Initially, a fit to eq 2 was attempted by fixing the \( \tau_2 \) component to 8, 6, and 3 ps for the TX-100, DTAB, and SDS micelles, respectively, and excellent fits were obtained. For curcumin in the TX-100 micelle, the fast decay component in \( C(t) \) has a time constant of 0.31 ps, which is as short as the instrument response function (IRF) of our apparatus, and an amplitude of
of the corresponding fluorescence upconversion decay at 520 nm within the multiexponential function $C(t)$ is well-fitted with a biexponential 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; see Figure 3 and Table 1.

**TABLE 2: Solvation Correlation Function, $C(t)$, Decay Parameters for Curcumin in Different Micellar Systems**

<table>
<thead>
<tr>
<th>Micellar System</th>
<th>$f_{300fs}$</th>
<th>$a_1$</th>
<th>$\tau_1$ (ps)</th>
<th>$a_2$</th>
<th>$\tau_2$ (ps)</th>
<th>$a_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX-100</td>
<td>0.25 ± 0.03</td>
<td>0.37 ± 0.02</td>
<td>0.31 ± 0.04</td>
<td>0.22 ± 0.03</td>
<td>8</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>DTAB</td>
<td>0.53 ± 0.03</td>
<td>0.56 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>0.20 ± 0.02</td>
<td>6</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>SDS</td>
<td>0.50 ± 0.03</td>
<td>0.54 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.26 ± 0.02</td>
<td>3</td>
<td>0.20 ± 0.01</td>
</tr>
</tbody>
</table>

$^a f_{300fs}$: 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 $\tau_2$ 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 Figure 3 and Table 1.

37%. In the DTAB and SDS micelles, however, the time constants of the fast component have values of 0.12 and 0.15 ps, respectively, which are shorter than the IRF of the instrument. These IRF-limited decay time constants have amplitudes of 56 and 54% in these two micelles. The fast component 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%. In addition to the fast (<300 fs) and slow (3–8 ps) components of $C(t)$, it is obvious that solvation dynamics are incomplete within the time window of the experiment, giving rise to a constant offset in the $C(t)$, as shown in Figure 5, and the $a_3$ term in the multiexponential equation in Table 2. Alternatively, a good fit to eq 2 can also be obtained by fixing the fast component to 0.1 ps for the three micelles. In this case, the slow component has time constants of 1.8, 2.6, and 1.9 ps, respectively. The fitting parameters are provided in the Supporting Information. The significance of the decay components of $C(t)$ are discussed below.

Solvation dynamics in micellar media is currently an intense area of research. Surfactant micelles are excellent model systems for more complex biomembranes, and investigations on solvation dynamics in micelles provide critical insight into the behavior of small molecules in biomembranes. First of all, in our study, it is noteworthy that for each of the micelles, the value of the time constant $\tau_2$ of $C(t)$, as summarized in Table 2, is virtually identical within experimental error to that of the fast component of the fluorescence upconversion results shown in Figure 3 and Table 1. The excellent agreement strongly indicates that the fast component observed in the fluorescence upconversion results (Figure 3 and Table 1) is due to solvation dynamics of curcumin in micellar media. Second, the decay of $C(t)$ of curcumin in all micelles show a fast component with a time constant that is either as fast as the IRF (300 fs) or too short to be resolved with our apparatus. This fast decay component was also reported for the SDS and DTAB micelles in a study by Dey et al. using coumarin 480 as the probe molecule, and a time constant of <300 fs was measured. This fast component of solvation dynamics has been previously attributed to motions of labile or bulk-like water molecules at the interface of the micelle. As for fast solvation dynamics in TX-100, Mandal et al. showed that the fastest component has a time constant of 2.1 ps, which is somewhat longer than our measured value of ~300 fs. To our knowledge, a ~300 fs solvation dynamics component in the TX-100 micelle is previously unobserved, and our result is the first demonstration of ultrafast solvation dynamics of the TX-100 micelle. Third, it is apparent that curcumin has a shorter time constant of fast solvation dynamics in the SDS/DTAB micelles (<300 fs) than that in the TX-100 micelle (~300 fs). This phenomenon is likely to originate from the level 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 micelles is substantially thinner (6–9 Å) than that of the TX-100 micelle (20 Å). This result indicates that curcumin is less shielded from the bulk-like water molecules in the former two micelles than that in the latter, and hence faster solvation dynamics are observed in the SDS and DTAB micelles. Fourth, the slow component of $C(t)$, which has time constants of 8, 6, and 3 ps, respectively, in the TX-100, DTAB, and SDS micelles, shows reasonable agreement with results from previous studies, which has been assigned to solvation of water molecules that are bound to the surface of the micelle by hydrogen bonding or the head group–water interactions. Lastly, the constant offset observed in the $C(t)$ of curcumin in the three micelles, which is expressed as $a_3$ in Table 2, indicates the presence of a decay component with a time constant that is vastly longer than the 10 ps time window of the solvation dynamics studies. Although this decay component of $C(t)$ is too long to be resolved with our solvation dynamics investigations, 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.

**Fluorescence Lifetime in Micelles.** The excited-state kinetics of curcumin in micelles was also investigated using time-correlated single-photon counting (TCSPC). Figure 6 shows the fluorescence decays of curcumin in the TX-100, DTAB, and SDS micelles in tris (pH 7.4) and tris-d$_4$ (pD 7.4) buffer with a 3.33 ns time window, in which curcumin produces an isotope effect in each of the micelles. The decays are multieponential in nature, and three exponential functions are necessary to fit the data well. The best-fit parameters and the average fluorescence lifetimes are summarized in Table 3. Briefly, in H$_2$O, the fastest decay component has a time constant ranging from 50 to 60 ps, which is followed a slower component of ~200 ps and then a long-lived 400–900 ps component. In contrast, in D$_2$O, while the two slower components are essentially indistinguishable within experimental error, the fastest component increases to 70–85 ps, exhibiting an isotope effect of 1.4 in the three micelles.

It is noteworthy that there is a close match between the time constants of the ESIHT component, as established in an earlier section on fluorescence upconversion, and the $\tau_1$ component as
Fluorescence decays obtained with time-correlated single-photon counting for curcumin in (A) TX-100, (B) DTAB, and (C) SDS micelle at pH = 7.4 (red) and pH = 7.4 (blue) tris buffer. The decays were fitted with the multiexponential function $f(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\tau_3)$, where $a_1 + a_2 + a_3 = 1$. The $\tau_2$ values were limited to the range specified.

### TABLE 3: Fluorescence Lifetime Results of Curcumin in Different Micellar Systems

<table>
<thead>
<tr>
<th>Micellar System</th>
<th>$a_1$ ($\pm$)</th>
<th>$\tau_1$ (ps)</th>
<th>$a_2$</th>
<th>$\tau_2$ (ps)</th>
<th>$a_3$</th>
<th>$\tau_3$ (ps)</th>
<th>$(\tau)$ (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX-100/H$_2$O</td>
<td>0.68 ± 0.02</td>
<td>60 ± 5</td>
<td>0.30 ± 0.02</td>
<td>210 ± 30</td>
<td>510 ± 100</td>
<td>115 ± 15</td>
<td></td>
</tr>
<tr>
<td>TX-100/D$_2$O</td>
<td>0.48 ± 0.02</td>
<td>85 ± 5</td>
<td>0.32 ± 0.02</td>
<td>210 ± 30</td>
<td>430 ± 20</td>
<td>195 ± 12</td>
<td></td>
</tr>
<tr>
<td>DTAB/H$_2$O</td>
<td>0.88 ± 0.02</td>
<td>50 ± 5</td>
<td>0.11 ± 0.02</td>
<td>190 ± 50</td>
<td>900 ± 200</td>
<td>75 ± 12</td>
<td></td>
</tr>
<tr>
<td>DTAB/D$_2$O</td>
<td>0.72 ± 0.03</td>
<td>70 ± 5</td>
<td>0.27 ± 0.03</td>
<td>190 ± 50</td>
<td>730 ± 300</td>
<td>110 ± 17</td>
<td></td>
</tr>
<tr>
<td>SDS/H$_2$O</td>
<td>0.91 ± 0.01</td>
<td>55 ± 5</td>
<td>0.08 ± 0.01</td>
<td>185 ± 10</td>
<td>415 ± 80</td>
<td>70 ± 6</td>
<td></td>
</tr>
<tr>
<td>SDS/D$_2$O</td>
<td>0.55 ± 0.02</td>
<td>75 ± 5</td>
<td>0.44 ± 0.02</td>
<td>185 ± 10</td>
<td>405 ± 20</td>
<td>125 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

A The fluorescence decay traces, $f(t)$, were fitted with the multiexponential function $f(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\tau_3)$, where $a_1 + a_2 + a_3 = 1$. The $\tau_2$ values were limited to the range specified.

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Supporting Information Available: Supplementary results on time-resolved emission spectra and alternative fitting parameters for $C(t)$. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


DTAB, and SDS micelles. The fluorescence upconversion transient of curcumin in each micelle shows a biexponential decay with time constants of 3–8 ps (fast) and 50–80 ps (slow). The slow component exhibits a pronounced isotope effect, producing a decay time constant of 80–130 ps in the micelles, which is assigned to ESHT. The ESHT rate of curcumin in the TX-100 micelle is lower than those in the other two micellar system. The hydrogen bonding between curcumin and the TX-100 surfactant may contribute to this effect. The fast decay component, unlike the ESHT process, is insensitive to deuterium of curcumin and has been attributed to solvation dynamics using results from multilength fluorescence upconversion studies. The water molecules in the micellar structure give rise to solvation dynamics of curcumin. The solvation dynamics observed in the micelles are due to water molecules at the micelle interface that are labile or bulk-like and those that are bound to the surface of the micelles.

Conclusion

We have presented unambiguous results to demonstrate that excited-state intramolecular hydrogen atom transfer (ESHT) is a major photophysical process of curcumin in the TX-100,