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Femtosecond Transient Absorption Spectroscopy of the Medicinal Agent Curcumin in Diamide Linked γ-Cyclodextrin Dimers

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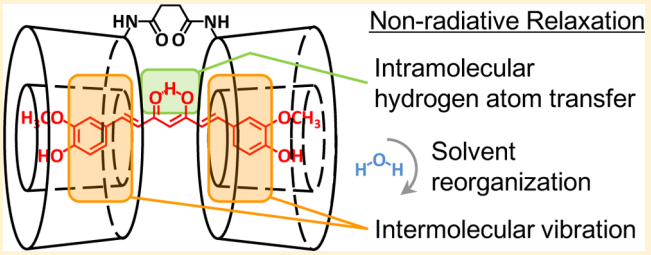
ABSTRACT: Curcumin is a biologically active polyphenol and a yellow pigment extracted from turmeric. Our previous study has shown effective encapsulation of curcumin using diamide linked γ-cyclodextrin dimers, namely 66γCD$_2$su and 66γCD$_2$ur, through cooperative 1:1 host-guest complexation. In this study, the excited-state dynamics of curcumin complexed with either 66γCD$_2$su or 66γCD$_2$ur in water are investigated using femtosecond transient absorption spectroscopy. Both 66γCD$_2$su-curcumin and 66γCD$_2$ur-curcumin complexes in water show only an excited-state absorption (ESA) band at 530 nm without any stimulated emission (SE) signals, indicating non-radiative decays as the major relaxation pathways. The ESA dynamics of 66γCD$_2$su-curcumin are similar to those of 66γCD$_2$ur-curcumin, consisting of a rapid growth component and three decay components. The growth component, which has a time constant of 0.25−0.41 ps, is assigned to solvent reorganization. The relatively fast decay components with time constants of 9.3−21.8 ps show significant deuterium isotope effect, consistent with the presence of excited-state intramolecular hydrogen atom transfer (ESIHT) of curcumin. The small-amplitude and slow decay components may be attributed to the dynamics of complexed curcumin and molecular motions due to flexibility of 66γCD$_2$su and 66γCD$_2$ur. In addition, transient absorption anisotropy measurements reveal slow rotational motions of 66γCD$_2$su-curcumin and 66γCD$_2$ur-curcumin complexes. The overall results show that complexation in 66γCD$_2$su and 66γCD$_2$ur has pronounced effects on the photophysics of curcumin.



INTRODUCTION

Curcumin (Figure 1a) is a naturally occurring yellow polyphenol present in the rhizomes of the spice plant *Curcuma longa*, commonly known as turmeric.[1,2] It constitutes 77% of curcuminoids, which are composed of a group of curcumin analogues, in company with demethoxycurcumin (17%) and bisdemethoxycurcumin (3%).[3] Another curcuminoid, cyclocurcumin, which is also present but at a much lower level,[4] was recently studied.[5] The dominant conformation of curcumin is the keto-enol form in polar solvents (Figure 1a).[1,2] Recent research on curcumin has shown its medicinal effects, including anticancer,[6−10] anti-Alzheimer's disease,[11−13] anti-cystic fibrosis,[14,15] and anti-inflammation properties.[16] However, the poor aqueous stability and solubility of curcumin limit bioavailability and hence hinder applications of curcumin as an effective therapeutic drug.[17−19] Therefore, molecular assemblies as delivery systems, which include micelles,[20−23] globular proteins,[24,25] polymer nanoparticles,[26,27] micelle-like aggregates and hydrogels,[28] and cyclodextrins,[29,30] have been developed and investigated to improve the availability of curcumin in vivo.

Cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides with either 6 (α), 7 (β), or 8 (γ) glucopyranoside units. Figure 1b shows the structure of γ-CD. The hydrophobic interior of CDs has the ability to encapsulate a hydrophobic molecular species, while the hydrophilic exterior allows the CD-drug host-guest complexes to be suspended in water, which is attractive for delivering a hydrophobic drug.[31,32] However, this approach is limited to small molecule drugs due to the limited cavity size of CDs.[33] In order to further improve the CD-based drug delivery systems, a diamide linker between two CDs has been introduced to promote cooperative binding to large molecular guests.[34−36] Our previous study has shown a strong 1:1 cooperative binding of diamide linked γ-CD dimers, namely 66γCD$_2$su and 66γCD$_2$ur, to curcumin (Figures 1c and 1d), which results in a remarkable aqueous stability of curcumin under physiological conditions.[29]

Our recent study has also reported the use of 66γCD$_2$su and 66γCD$_2$ur as curcumin delivery systems to cancer cells,[6] and other studies have shown that curcumin has a range of phototherapeutic effects on melanoma cells.[37−39] There is a growing interest in understanding the photophysical processes of curcumin.[40−47] Thus far, femtosecond transient absorption spectroscopy and fluorescence upconversion spectroscopy have shown that the dominant relaxation pathway of curcumin is excited-state intramolecular hydrogen atom transfer (ESIHT).[40−45] The ESIHT process is influenced by polar solvents including methanol, DMSO, and acetone due to solvent-curcumin interactions. Although there have been reports of a relatively fast ESIHT of curcumin in nonpolar

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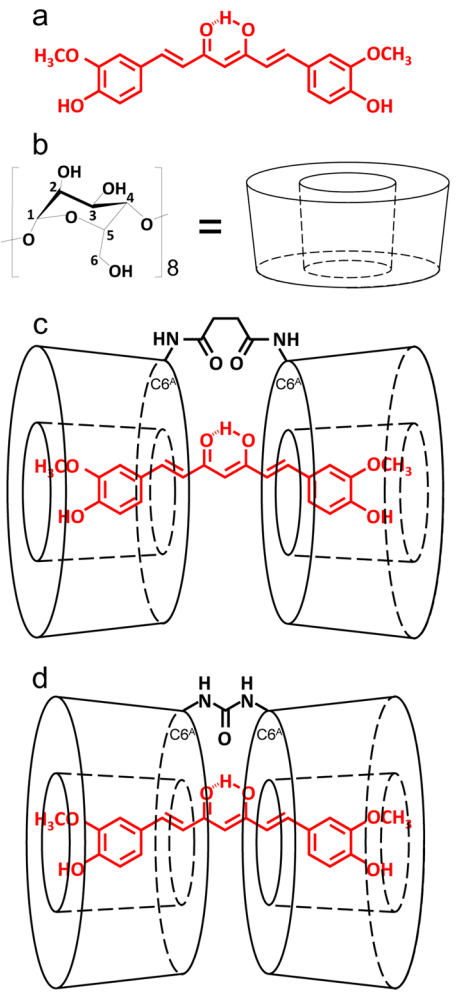

Figure 1. Structures of (a) curcumin, (b) γ-CD, and curcumin complexed with (c) 66γCD_2su and (d) 66γCD_2ur.

solvent systems,[43,44,47] it is still highly unlikely that the observed ESIHT occurs in a direct manner, possibly due to the presence of trace quantities of water in these solvents. Previous work on 3-hydroxyflavone showed that trace amounts of hydrogen-bonding substances can have a significant effect on ESIHT.[48] Furthermore, Schwartz et al. showed that even with rigorous purification of methylcyclohexane traces of hydrogen-bonding impurities (e.g., H_2O) are still present, affecting ESIHT of 3-hydroxyflavone.[49] Nevertheless, the study by Schwartz et al. revealed that ESIHT of 3-hydroxyflavone occurs with a time constant of 240 fs in a highly purified and dried environment.[49] This work provided the first insight into the ultrafast timescale for a direct ESIHT. Interestingly, a very recent study by Mohammed et al. showed that ESIHT of 1,8-dihydroxy-9,10-anthraquinone (DHAQ) involves a direct hydrogen atom transfer from an enol to a keto group in a wide range of solvents.[50] The results suggest that ESIHT of DHAQ occurs within the first 150 fs of the dynamics. Therefore, it follows that a direct ESIHT of curcumin should occur with a similar time constant to those of 3-hydroxyflavone and DHAQ. In addition to the timescale of ESIHT, there have been some debates on whether the excited-state hydrogen atom transfer reaction of curcumin is intramolecular or intermolecular in nature in polar protic solvents.[41,42,47] While Adhikary et al. proposed an intramolecular pathway,[41,42] Ghosh et al. suggested intermolecular hydrogen-bonding of curcumin with the polar protic solvent as an excited-state hydrogen atom transfer mechanism.[47] Although it appears that there are two different pathways, these two proposals are in fact very similar, if not identical. This is because the intramolecular pathway proposed by Adhikary et al. actually involves interactions of curcumin with the polar protic solvent, which is consistent with the conclusions drawn by Kasha and Maroncelli on the ESIHT reactions of 3-hydroxyflavone and 7-azaindole, respectively.[48,51] Therefore, rather than having two different mechanisms for excited-state hydrogen atom transfer, there is in fact a consensus in the field that interactions with polar protic solvents play an important role in ESIHT of curcumin.

Here, we report the excited-state photodynamics of curcumin complexed by the cyclodextrin dimers, 66γCD_2su and 66γCD_2ur, for the first time. The steady-state absorption and fluorescence spectra show that curcumin is nonfluorescent when it is complexed by 66γCD_2su or 66γCD_2ur. Femtosecond transient absorption spectroscopy reveals excited-state absorption of curcumin complexed by 66γCD_2su or 66γCD_2ur in water, without any stimulated emission signals. The nonradiative decay processes involve ESIHT of curcumin, reorganization of water molecules within the cavity of 66γCD_2su and 66γCD_2ur, and other processes including dynamics of complexed curcumin and molecular motions due to flexibility of the γ-CD moieties of 66γCD_2su and 66γCD_2ur. Time-resolved anisotropy results show that the rotational motions of curcumin complexed by 66γCD_2su and 66γCD_2ur occur on a significantly longer timescale than that of curcumin in a polar organic solvent. Overall, the femtosecond transient absorption results provide insight into the photophysical properties of curcumin when it is complexed by 66γCD_2su and 66γCD_2ur.

MATERIALS AND METHODS

Materials. Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) was obtained from LKT Laboratories (purity >98%). A previous study showed that curcumin with this purity was still comparable to that with a 70% purity,[42] and hence, the 2% impurities were unlikely to affect our spectroscopic measurements. Methanol (MeOH, HPLC grade, 99.7%) from Scharlau was used as received, and deuterated water (D_2O) and methanol (MeOD-d_4) were purchased from Cambridge Isotope Laboratories (D, 99.9%). Water (H_2O) was obtained from a Millipore Milli-Q NANOpure water system. The pH of H_2O and pD of D_2O were approximately 7. The $C6^A$-to-$C6^A$ diamide linked γ-cyclodextrin dimers, *N,N′*-bis(6^A-deoxy-γ-cyclodextrin-6^A-yl) succinamide (66γCD_2su), and *N,N′*-bis(6^A-deoxy-γ-cyclodextrin-6^A-yl) urea (66γCD_2ur), were synthesized using literature methods.[36] Briefly, the native γ-CDs were substituted with 4-toluenesulfonyl chloride for activation at the $C6^A$ position, which yielded 6^A-*O*-(4-methylbenzenesulfonyl)-γ-cyclodextrin (6γCDTs). For the synthesis of 66γCD_2su, the reaction between 6γCDTs and ammonium bicarbonate produced 6^A-amino-6^A-deoxy-γ-cyclodextrin, 6γ$CDNH_2$, which was then dimerized by the reaction with bis(4-nitrophenyl) succinate as the linker. For the synthesis of 66γCD_2ur, the reaction between 6γCDTs and sodium azide produced 6^Aazido-6^A-deoxy-γ-cyclodextrin, 6γCDN_3, which was then dimerized by reaction with carbon dioxide as the linker.

Steady-State UV-Visible Absorption and Fluorescence Spectroscopic Studies. UV-visible absorption spectra of 1 μM curcumin complexed with either 66γCD_2su or 66γCD_2ur in

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a 1:1 ratio in H_2O or D_2O in a 1-cm quartz cuvette were recorded from 300 to 700 nm using a Cary 5000 UV-Vis/NIR spectrophotometer (Varian). Similarly, UV-visible absorption spectra of 1 μM curcumin in either MeOH or MeOD-d_4 were acquired from 300 to 700 nm. Fluorescence spectra of these solutions were subsequently recorded from 415 to 700 nm using a Cary Eclipse Fluorescence spectrophotometer (Varian) with the excitation and emission slit widths set at 5 nm. The excitation wavelength for these experiments was set at 400 nm. The reported spectra were averaged over 5 scans at a scan rate of 600 nm/min.

Femtosecond Transient Absorption Spectroscopic Studies. Solutions of 80 μM curcumin complexed with either 66$\gamma$$CD_2$su or 66$\gamma$$CD_2$ur in a 1:1 ratio in H_2O or D_2O were used in the transient absorption spectroscopic studies. Similarly, a curcumin concentration of 80 μM was used in MeOH or MeOD-d_4. All the measurements were acquired using a quartz cuvette with a 2-mm path length. Less than 10% of curcumin photodegradation was observed after each set of data acquisition.

The laser system used for the femtosecond transient absorption experiments consisted of a Ti:sapphire mode-locked oscillator (Spectra-Physics, Tsunami), which seeded a Ti:sapphire regenerative amplifier (Spectra-Physics, Spitfire Pro XP) pumped by a 20W Q-switched Nd:YLF laser (Spectra-Physics, Empower). The output of the amplifier was centered at 800 nm with a repetition rate of 1 kHz and pulse duration of 100 fs, which was then split into pump and probe beamlines. The 400-nm pump pulses were generated using a type-I BBO crystal (Eksma Optics), which was modulated at 500 Hz and then focused onto the sample with a spot size of 710 μm and pulse energy of 720 nJ. The probe beam passed through a delay stage and was used to generate a white light continuum in a 2-mm sapphire crystal. The probe passed through a beam splitter to produce the sample and reference beams with a spot size of 50 μm at the sample position. The sample and reference beams were then directed into complementary CMOS detectors for detection in the visible region. The probe polarization was oriented at magic angle (54.7°) with respect to the pump polarization. In all the measurements, a full width at half maximum (fwhm) of 100 fs was used for data analysis.

The same laser system was utilized for the femtosecond transient absorption anisotropy study. The measurement involved collecting two transient absorption signals with the probe polarization at 0° (parallel, $I_{\parallel}$) and 90° (perpendicular, $I_{\perp}$) with respect to the pump polarization, in order to determine the time-dependent anisotropy $r(t)$ (see eq 1). In this study, a concentration of 160 μM was used for 66$\gamma$$CD_2$su or 66$\gamma$$CD_2$ur while the concentration of curcumin was kept at 80 μM curcumin to minimize the probability of multiple curcumin molecules binding to a single diamide linked γ-CD dimer.

RESULTS AND DISCUSSION

Steady-State Absorption and Fluorescence Spectra of Curcumin Complexed in 66$\gamma$$CD_2$su and 66$\gamma$$CD_2$ur. The steady-state absorption and fluorescence spectra of the 66$\gamma$$CD_2$su-curcumin and 66$\gamma$$CD_2$ur-curcumin complexes are displayed in Figures 2a and 2b, respectively. The spectral envelopes of the curcumin complexes in H_2O and D_2O are very similar except for a minor blue shift for the D_2O solution. In comparison with H_2O and D_2O solvent systems, Figure 2c shows the steady-state UV−visible absorption and fluorescence

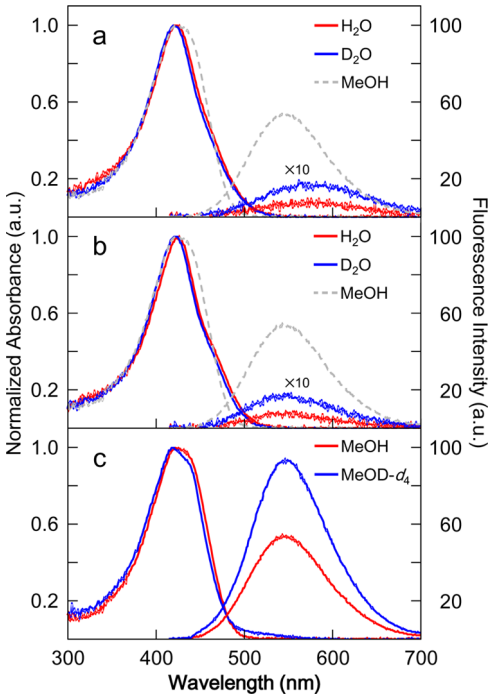

Figure 2. Steady-state absorption and fluorescence spectra of curcumin complexed with (a) 66$\gamma$$CD_2$su and (b) 66$\gamma$$CD_2$ur in H_2O (red) and D_2O (blue), and (c) curcumin in MeOH (red) and MeOD-d_4 (blue), respectively. The gray dashed spectra in (a) and (b) represent steady-state absorption and fluorescence spectra of curcumin in MeOH as a reference. The fluorescence spectra of 66$\gamma$$CD_2$su-curcumin or 66$\gamma$$CD_2$ur-curcumin complexes in H_2O or D_2O were multiplied by ten for illustration purposes.

spectra of curcumin in MeOH (red) and MeOD-d_4 (blue). The absorption peak around 430 nm corresponds to the $\pi-\pi^*$ transition of the keto-enol tautomer of curcumin (Figure 1a).[20,42] The absorption spectrum of curcumin in MeOD-d_4 exhibits a very small blue shift without observable spectral changes.[52] The absorption peak around 420 nm and spectral envelope of 66$\gamma$$CD_2$su-curcumin and 66$\gamma$$CD_2$ur-curcumin are similar to those of curcumin in MeOH, which are shown as a gray dashed spectrum in Figures 2a and 2b. This result indicates that the keto-enol tautomer of curcumin is present in the 66$\gamma$$CD_2$su-curcumin and 66$\gamma$$CD_2$ur-curcumin complexes, as shown in Figures 1c and 1d.[29]

Additionally, the absorption spectra of both 66$\gamma$$CD_2$su-curcumin and 66$\gamma$$CD_2$ur-curcumin complexes show no 350-nm spectral shoulder, indicating a lack of significant water-curcumin interactions.[20,24,29] However, the water-curcumin interactions are still sufficiently strong such that the 66$\gamma$$CD_2$su-curcumin and 66$\gamma$$CD_2$ur-curcumin complexes exhibit very weak fluorescence signals (Figures 2a and 2b).[29] At the same curcumin concentration, the fluorescence spectrum of curcumin in MeOD-d_4 exhibits a higher intensity than that of curcumin in MeOH, while their spectral envelopes remain similar (Figure 2c).[52] The fluorescence intensities of 66$\gamma$$CD_2$su-curcumin and 66$\gamma$$CD_2$ur-curcumin in D_2O are also higher than those in H_2O. It is clear that the fluorescence intensity and hence the fluorescence quantum yield of curcumin is sensitive to the hydrogen/deuterium (H/D) exchange of curcumin.

It has been shown previously that the enolic hydrogen of curcumin undergoes H/D exchange in deuterated protic solvents and changes the fluorescence quantum yields of

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curcumin.[52] A previous time-resolved fluorescence spectroscopic study has concluded that ESIHT is a major photophysical process of curcumin in polar protic solvents.[42] The higher fluorescence quantum yield of curcumin in MeOD-d_4 than that in MeOH is due to a longer fluorescence lifetime as a result of the deuterium isotope effect.[42,52] Although time-resolved fluorescence spectroscopy has been very useful in previous work, it is inapplicable to 66γCD$_2$su-curcumin and 66γCD$_2$ur-curcumin due to their nonfluorescent nature.[29] Hence, in this study, transient absorption spectroscopy was used to offer insight into the excited-state dynamics of the 66γCD$_2$su-curcumin and 66γCD$_2$ur-curcumin complexes.

Femtosecond Transient Absorption of Curcumin Complexed in 66γCD$_2$su and 66γCD$_2$ur. Femtosecond transient absorption spectroscopy has been employed for investigations of the excited-state dynamics of curcumin in different systems.[40,47] Here we report the first femtosecond transient absorption study on curcumin solely complexed in 66γCD$_2$su and 66γCD$_2$ur, in a 1:1 host:guest ratio in the aqueous environment. Figures 3a and 3b show the transient absorption spectra of the 66γCD$_2$su-curcumin complex in H_2O and D_2O, respectively, with several probe delay times, ranging from 0.1 to 200 ps. The excited-state absorption (ESA) band of curcumin complexed in 66γCD$_2$su in H_2O at 0.1 ps shows a 500-nm peak, but the 530-nm ESA signal becomes pronounced at >1 ps. Figures 3a and 3b show that the transient absorption dynamics of 66γCD$_2$su-curcumin in H_2O are faster than those in D_2O, which is discussed below. A similar behavior in the transient absorption spectra are also present when curcumin is complexed with 66γCD$_2$ur in H_2O or D_2O, as shown in Figures 3c and 3d, respectively.

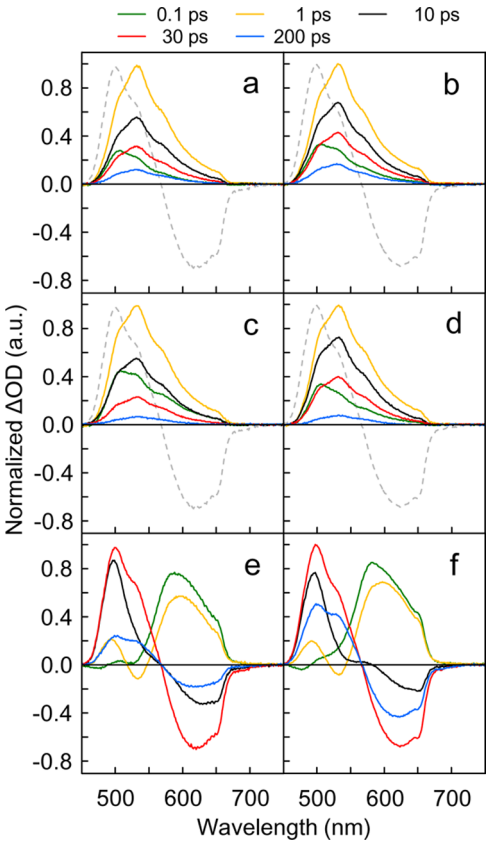

Figure 3. Transient absorption spectra of 66γCD$_2$su-curcumin complex in (a) H_2O and (b) D_2O, those of 66γCD$_2$ur-curcumin complex in (c) H_2O and (d) D_2O, and those of curcumin in (e) MeOH and (f) MeOD-d_4, at different time delays. Each set of spectra is normalized to the maximum ΔOD signal of the sample. The gray dashed spectrum in (a–d) represents transient absorption spectra of curcumin in either (e) MeOH or (f) MeOD-d_4 at 30 ps as a reference.

Figures 3e and 3f show the transient absorption spectra of curcumin in MeOH and MeOD-d_4, respectively, with several probe delay times ranging from 0.1 to 200 ps. Curcumin in MeOH exhibits a rapid ESA peak around 600 nm at 0.1 ps, which is followed by the appearance of another ESA peak around 500 nm and a stimulated emission (SE) signal around 540 nm at 1 ps. These early time ESA and SE peaks of curcumin in MeOH were also observed in a previous study.[40] The ESA at 600 nm rapidly evolves into SE between 10 and 200 ps. The SE signal observed at 540 nm becomes a part of the ESA band centered at 500 nm around 10 ps. This band reaches a maximum ΔOD value around 30 ps and decreases thereafter.

There are three notable differences between the transient absorption spectra of *complexed* and *free* curcumin. First, the transient absorption spectra of 66γCD$_2$su-curcumin and 66γCD$_2$ur-curcumin complexes only exhibit ESA signals within the time window of investigation. The transient absorption spectrum of curcumin in MeOH and MeOD-d_4 at 30 ps are shown in Figures 3a–3d as gray dashed spectra to highlight the absence of SE signals of curcumin complexed in 66γCD$_2$su and 66γCD$_2$ur. The absence of SE signals for the 66γCD$_2$su-curcumin or 66γCD$_2$ur-curcumin complexes is consistent with the very weak fluorescence signals, as shown in Figures 2a and 2b. Second, there is an apparent difference in the maximum ESA peak position between complexed and free curcumin. Curcumin complexed in either 66γCD$_2$su or 66γCD$_2$ur only shows an ESA peak at 530 nm. In contrast, the ESA maximum of free curcumin appears at 500 nm. However, the ESA and SE signals of free curcumin overlap between 450 and 700 nm such that the overall ESA maximum of free curcumin appears at 500 nm. Finally, the ESA signals of the 66γCD$_2$su-curcumin or 66γCD$_2$ur-curcumin complexes evolve faster than that of curcumin in methanol, as is discussed below.

Excited-State Dynamics of Curcumin Complexed in 66γCD$_2$su and 66γCD$_2$ur. Figures 4a and 4b show the 500-nm ESA signals of curcumin complexed with 66γCD$_2$su and 66γCD$_2$ur, respectively, in H_2O (red) and D_2O (blue) as a function of pump-probe delay time. The 500-nm ESA signals of 66γCD$_2$su-curcumin and 66γCD$_2$ur-curcumin complexes were fitted with a quad-exponential function consisting of a growth component (τ_1) and three decay components (τ_2, τ_3, and τ_4), which are summarized in Table 1. The ESA signals of 66γCD$_2$su-curcumin complex in H_2O and D_2O show a rapid growth component with a time constant (τ_1) of 0.25 ps with an amplitude of approximately ~40% of the maximum signal amplitude. Comparable results were obtained for the 66γCD$_2$ur-curcumin complex (Table 1). There are three decay components characterized by time constants, τ_2, τ_3, and τ_4, for the ESA signals of both the 66γCD$_2$su-curcumin and 66γCD$_2$ur-curcumin complexes (Table 1). The time constants τ_2 are approximately 9.3 ps (39%) and 14.6 ps (34%) for the 66γCD$_2$su-curcumin complex in H_2O and D_2O, respectively. For the 66γCD$_2$ur-curcumin complex in H_2O and D_2O solvent systems, the time constants τ_2 are 12.7 ps (55%) and 21.8 ps (53%), respectively. The time constants τ_3 and τ_4 are fixed to 109 and 1200 ps, respectively,[53] which are discussed below.

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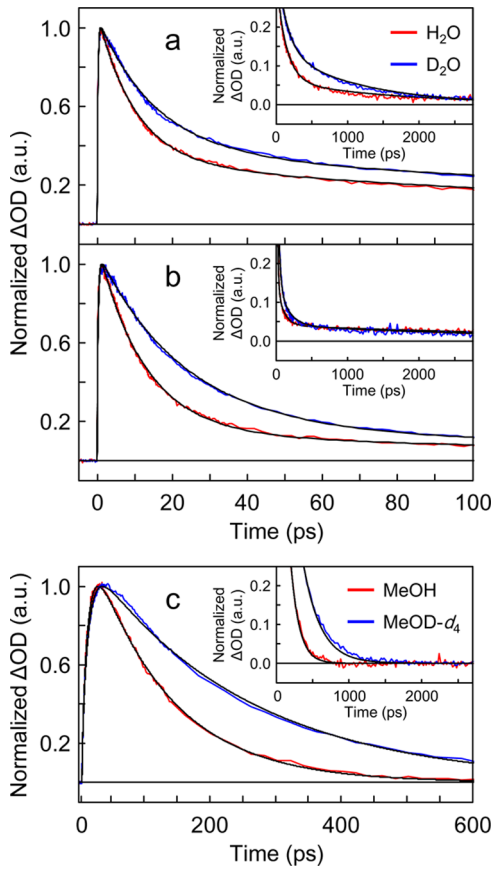

Figure 4. Normalized excited-state absorption signals of (a) 66γCD_2su-curcumin and (b) 66γCD_2ur-curcumin complexes in H_2O (red) and D_2O (blue), and (c) those of curcumin in MeOH (red) and MeOD-d_4 (blue), at 500 nm. The insets show the signals at later times. Note the timescales of (a) and (b) are different from that of (c).

The rapid growth components τ_1 of the ESA signal for both 66γCD_2su-curcumin and 66γCD_2ur-curcumin complexes are assigned to reorganization of H_2O and D_2O,[41,53,54] which is consistent with the Stokes shift observed in the steady-state spectra (Figures 2a and 2b). Vajda et al. have investigated the dynamics of water molecules inside the γ-CD cavity, and their results indicate the presence of significant solute-solvent interactions inside the γ-CD cavity, occurring within 1 ps.[53] Therefore, the interactions between curcumin and water molecules inside and cavities are expected. The presence of water-curcumin interactions is consistent with the non-fluorescent nature of 66γCD_2su-curcumin and 66γCD_2ur-curcumin complexes,[29] and the results from a previous molecular dynamics study on these complexes.[55] The process with a time constant of τ_2 is the dominant relaxation process and shows a deuterium isotope effect of 1.6 for 66γCD_2su-curcumin and 1.7 for 66γCD_2ur-curcumin. This relaxation is attributable to ESIHT, which is in agreement with previous studies on curcumin in protic solvents including methanol.[41,42] The substantial contribution of the decay component τ_2 (approximately 35−55%) indicates that ESIHT is an important photophysical process of excited-state curcumin complexed with 66γCD_2su and 66γCD_2ur. However, the resultant deuterium isotope effect of τ_2 is less than 2, indicating that other relaxation processes may occur at similar timescales to ESIHT. The relative amplitude of the ESIHT component is lower for 66γCD_2su-curcumin (37%) than 66γCD_2ur-curcumin (54%), as shown in Table 1. The difference in relative amplitude is likely to be related to the difference in the hydrogen-bonding interactions of curcumin in these assemblies. A recent computational study suggested a significant level of hydrogen bonding between curcumin and the diamide linker of 66γCD_2ur.[55] However, this interaction is absent between curcumin and 66γCD_2su due to the high flexibility of the diamide linker. The long decay time constants τ_3 and τ_4 are present in the ESA decay without showing any deuterium isotope effect. These small-amplitude and slow relaxation processes may involve motions of complexed curcumin and the γ-CD moieties of 66γCD_2su and 66γCD_2ur due to their flexibility. A previous study has shown that the limited cavity size of γ-CD leads to the appearance of small-amplitude and slow relaxation processes, including diffusive motions of the guest molecule, reorientation of highly constrained water molecules within the γ-CD cavity, or fluctuations of the γ-CD molecule.[53] In the same study, the time constants of 109 and 1200 ps were assigned to these molecular motions.[53] Here, we fixed τ_3 and τ_4 to these values as these molecular motions are expected to be present in both 66γCD_2su-curcumin and 66γCD_2ur-curcumin complexes. Similar results have also been reported in other studies.[56,57] Moreover, a recent computational study has shown the presence of water molecules and diffusive motions of curcumin within the γ-CD cavities of 66γCD_2su and 66γCD_2ur.[55] In addition, our previous 2D NOESY ^{1}H NMR spectroscopic results show the intermolecular alkyl hydrogen-hydrogen interactions between curcumin and the γ-CD moieties of 66γCD_2su and 66γCD_2ur.[29] In short, the slow relaxation dynamics of 66γCD_2su-curcumin and 66γCD_2ur-curcumin complexes may be derived from fluctuations of the γ-CD moieties, reorientation of constrained water molecules, diffusive motions of curcumin within the γ-CD

Table 1. Transient Absorption Kinetic Parameters of Curcumin in Different Systems at 500 nm[a]

host	solvent	a_1[b]	τ_1 (ps)	a_2[b]	τ_2 (ps)	a_3	τ_3[c] (ps)	$\langle\tau\rangle$[d] (ps)
66γCD_2su	H_2O	−0.42	0.25	0.39	9.3	0.14	109	126
66γCD_2su	D_2O	−0.44	0.25	0.34	14.6	0.14	109	210
66γCD_2ur	H_2O	−0.38	0.33	0.55	12.7	0.04	109	83
66γCD_2ur	D_2O	−0.36	0.41	0.53	21.8	0.08	109	103
–	MeOH	−0.06	1.17	−0.43	9.4	0.51	116	116
–	MeOD-d_4	−0.07	1.81	−0.42	10.6	0.51	231	231

[a]The transient absorption kinetics were fitted with the multiexponential function, $f(t) = \sum_{i=1}^{n} a_i \exp(-t/\tau_i)$, where $\sum_{i=1}^{n} |a_i| = 1$, with the minimum number of exponential terms (n). The relative error values of the parameters are approximately 10% from three independent measurements. [b]The negative amplitude signifies a growth component. [c]The τ_3 and τ_4 values are fixed to 109 and 1200 ps, respectively, for curcumin complexed in 66γCD_2su and 66γCD_2ur.[53] See text for details. [d]$\langle\tau\rangle$ is defined as $(\sum_{j=1}^{k} a_j\tau_j)/\sum_{j=1}^{k} a_j$ for the decay components (k).

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cavities, and/or alkyl hydrogen-hydrogen vibrational motions between curcumin and the γ-CD moieties.

Figure 4c shows the time-dependent 500-nm ESA signals of free curcumin in MeOH (red) and MeOD-d_4 (blue), which decay slower than those of curcumin complexed in 66γCD_2su and 66γCD_2ur. The 500-nm ESA signals of curcumin in MeOH and MeOD-d_4 are fitted with a triexponential function with two growth components and a single decay component, which are shown in Table 1. The two growth components, with time constants τ_1 of 1.17 ps (6%) and τ_2 of 9.4 ps (43%), are in agreement with previous studies.[40,47] The growth components in the ESA signals of curcumin in MeOD-d_4 result in time constants τ_1 of 1.81 ps (7%) and τ_2 of 10.6 ps (42%), which are similar to those for curcumin in MeOH. These time constants for curcumin in either MeOH or MeOD-d_4 are assigned to reorganization of solvent molecules. In addition to the growth components, the ESA signals of curcumin in MeOH show a single decay component with a time constant τ_3 of 116 ps (51%), as previously reported.[40,47] The decay component in the ESA signal of curcumin in MeOD-d_4 results in a time constant τ_3 of 231 ps, showing a significant deuterium isotope effect of 2.0. This decay component is related to ESIHT, as previously investigated using femtosecond fluorescence upconversion spectroscopy.[41,42] Furthermore, the ESIHT process is influenced by polar solvent molecules, including methanol, due to significant solvent-curcumin interactions, resulting in a longer time constant of free curcumin in MeOH than that of curcumin complexed in 66γCD_2su and 66γCD_2ur.

Overall, the non-radiative relaxation processes of curcumin complexed in 66γCD_2su and 66γCD_2ur are determined to be solvent reorganization, ESIHT, and other slow dynamics. For free curcumin in MeOH and MeOD-d_4, solvent reorganization and ESIHT are the non-radiative relaxation processes. In all cases, the ESIHT is a major relaxation process and it plays an important role in the photophysical properties of curcumin.

Anisotropy of Curcumin Complexed in 66γCD_2su and 66γCD_2ur. To gain further insight into the dynamics of curcumin, the rotational motions of curcumin complexed in 66γCD_2su and 66γCD_2ur were investigated and compared with that of free curcumin in methanol. Transient absorption anisotropy involves measurements of two transient absorption signals with the probe polarization at 0° (parallel, $I_\parallel$) and 90° (perpendicular, $I_\perp$) with respect to the pump polarization. The polarization anisotropy function, $r(t)$, is defined as follows.

$$r(t) = \frac{I_\parallel(t) - I_\perp(t)}{I_\parallel(t) + 2I_\perp(t)} \quad (1)$$

The anisotropy decay, $r(t)$, is fitted using a single exponential function,

$$r(t) = r_0 \exp(-t/\tau^{(r)}) \quad (2)$$

where r_0 and $\tau^{(r)}$ are the initial anisotropy value and molecular rotation time constant, respectively.

Figures 5a and 5b show the anisotropy decays of 66γCD_2su-curcumin and 66γCD_2ur-curcumin in H_2O, respectively. The 66γCD_2su-curcumin and 66γCD_2ur-curcumin in H_2O show r_0 = 0.33 ± 0.01 and 0.32 ± 0.01, respectively. The deviation from the theoretical limit of 0.4 in both cases may be due to rapid energy transfer from curcumin to either 66γCD_2su or 66γCD_2ur and/or to the surrounding solvent molecules. It is clear from Figures 5a and 5b that $\tau^{(r)}$ of 66γCD_2su-curcumin and 66γCD_2ur-curcumin in H_2O are significantly longer than

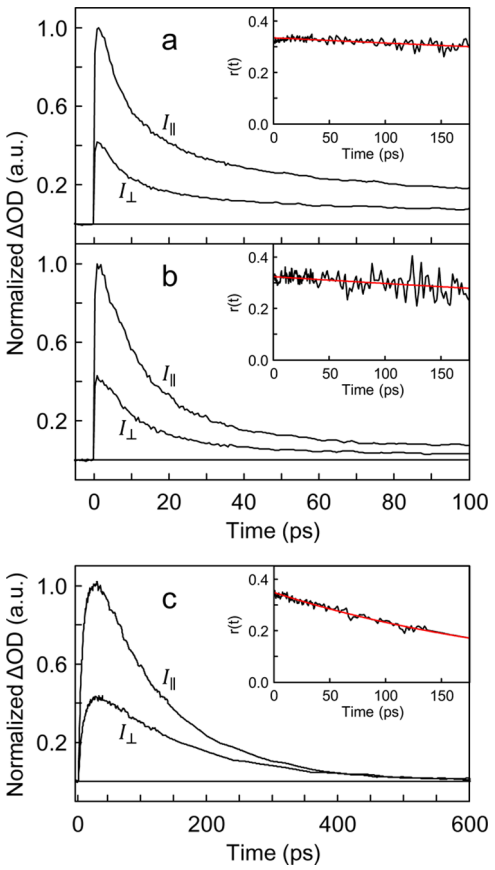

Figure 5. Transient absorption anisotropy decays of (a) 66γCD_2su-curcumin in H_2O, (b) 66γCD_2ur-curcumin in H_2O, and (c) curcumin in MeOH, at 500 nm. Note the timescales of (a) and (b) are different from that of (c). The insets show the anisotropy variations in the first 175 ps. Refer to eq 1 for the definitions of $I_\parallel(t)$ and $I_\perp(t)$.

the time window available for polarization anisotropy measurements. The $\tau^{(r)}$ value of >1200 ps produces a reasonable fit to $r(t)$ for these species. In contrast, Figure 5c shows the anisotropy decay of curcumin in MeOH, with r_0 = 0.34 ± 0.01 ps and $\tau^{(r)}$ = 249 ± 13 ps. These values are in good agreement with those from a previous study.[42] It is clear that rotational motions are present for curcumin in MeOH within the experimental time window. The slow anisotropy decays of 66γCD_2su-curcumin and 66γCD_2ur-curcumin indicate substantially slower rotational motions than that of curcumin in MeOH, as summarized in Table 2.

To understand the difference between the $\tau^{(r)}$ of curcumin in MeOH and in the diamide linked γ-CD dimers, we turn to the Debye-Stokes-Einstein relation to estimate the rotational time constant.

Table 2. Time-Resolved Transient Absorption Anisotropy Decay Parameters of Curcumin in Different Systems at 500 nm[a]

host	solvent	r_0	$\tau^{(r)}$ (ps)	$\tau^{(r)}_{DSE}$[b] (ps)
66γCD_2su	H_2O	0.33 ± 0.01	>1200	869−1189
66γCD_2ur	H_2O	0.32 ± 0.01	>1200	869−998
−	MeOH	0.34 ± 0.01	249 ± 13	375

[a]The transient absorption anisotropy decays were fitted to $r(t) = r_0 \exp(-t/\tau^{(r)})$. [b]Debye-Stokes-Einstein equation at 293 K (refer to eq 3).

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$$\tau_{DSE}^{(r)} = V\eta/k_BT \quad (3)$$

The symbols V, η, k_B, and T are the hydrodynamic volume of the species under investigation, the viscosity of the medium, the Boltzmann constant, and the temperature in Kelvin, respectively. With the use of $\eta_{MeOH} = 5.90 \times 10^{-4}$ Pa s at 293 K and a hydrodynamic radius of 0.85 nm for curcumin,[58] $\tau_{DSE}^{(r)}$ of curcumin in MeOH is 375 ps, which is in general agreement with the experimentally determined $\tau^{(r)}$ of ~250 ps. Similarly, with the use of the lengths of the succinamide and urea linkers and the dimensions of γ-CD,[33] the hydrodynamic volumes of 66γCD$_2$su and 66γCD$_2$ur are estimated to be $(3.50-4.79) \times 10^{-27}$ m^3 and $(3.50-4.02) \times 10^{-27}$ m^3, respectively, depending on the distances between the γ-CD moieties owing to flexibility of the diamide linkers. Therefore, given that $\eta_{H_2O} = 1.01 \times 10^{-3}$ Pa s at 293 K, the range of $\tau_{DSE}^{(r)}$ values for curcumin complexed in 66γCD$_2$su and 66γCD$_2$ur are 869−1189 and 869−998 ps, respectively. These $\tau_{DSE}^{(r)}$ values are also in general agreement with the experimentally determined $\tau^{(r)}$ values of >1200 ps. These results indicate that the substantially longer $\tau^{(r)}$ of 66γCD$_2$su-curcumin and 66γCD$_2$ur-curcumin complexes than that of curcumin in MeOH is due to the significantly larger hydrodynamic volumes of these complexes.

CONCLUSIONS

The excited-state dynamics of curcumin complexed by either 66γCD$_2$su or 66γCD$_2$ur in water are investigated for the first time, using femtosecond transient absorption spectroscopy. Comparisons are made with respect to *free* curcumin in methanol to gain insight into the effects of complexation in 66γCD$_2$su and 66γCD$_2$ur. Steady-state absorption and emission spectroscopic studies indicate significant reduction of water-curcumin interactions as a result of complexation in 66γCD$_2$su and 66γCD$_2$ur. Both 66γCD$_2$su-curcumin and 66γCD$_2$ur-curcumin in water only show an ESA signal at 530 nm without any SE signals, indicating the presence of significant non-radiative relaxation. The ESA dynamics of 66γCD$_2$su-curcumin and 66γCD$_2$ur-curcumin are similar, having a rapid growth component and three decay components. The growth component of 0.25−0.41 ps is assigned to rapid reorganization of solvent molecules. The relatively fast decay components are attributed to relaxation due to ESIHT (9.3−21.8 ps). The small-amplitude and slow decay components are likely to be due to dynamics of complexed curcumin and molecular motions due to flexibility of the γ-CD moieties of 66γCD$_2$su and 66γCD$_2$ur. Finally, the transient absorption anisotropy results reveal that the slow rotational motions of 66γCD$_2$su-curcumin and 66γCD$_2$ur-curcumin in comparison with that of curcumin in MeOH are due to their large hydrodynamic volumes.

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Notes

The authors declare no competing financial interest.

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