Sequestration Effect on the Open-Cyclic Switchable Property of Warfarin by Cyclodextrin: Ultrafast Dynamical Study

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ABSTRACT: The excited-state lifetimes of the anticoagulant drug warfarin (W) in water and in the absence and presence of methyl-*β*-cyclodextrins (Me-*β*-CD) were recorded using time-resolved fluorescence measurements. Selective excitation of the open and cyclic protonated isomers of W were acquired with laser emitting diodes (LED) producing 320 and 280 nm excitation pulses, respectively. Formation of the inclusion complex was checked by UV–visible absorption spectroscopy, and the values of binding constants (2.9×10^3 M^{-1} and 4.2×10^2 M^{-1} for protonated and deprotonated forms, respectively) were extracted from the spectrophotometric data. Both absorption and time-resolved fluorescence results established that the interior of the macromolecular host binds preferentially the open protonated form, red shifts the maximum of its absorption of light at ~305 nm, extends its excited-state lifetime, and decreases its emission quantum yield (Φ_F). Collectively, sequestration of the open guest molecules decreases markedly their radiative rate constants (k_r), likely due to formation of hydrogen-bonded complexes in both the ground and excited states. Due to lack of interactions, no change was observed in the excited-state lifetime of the cyclic form in the presence of Me-*β*-CD. The host also increases the excited-state lifetime and Φ_F of the drug deprotonated form (W^-). These later findings could be attributed to the increased rigidity inside the cavity of Me-*β*-CD. The pK_a values extracted from the variations of the UV–visible absorption spectra of W versus the pH of aqueous solution showed that the open isomer is more acidic in both ground and excited states. The positive shifts in pK_a values induced by three derivatives of cyclodextrins: HE-*β*-CD, Ac-*β*-CD, and Me-*β*-CD supported the preferential binding of these hosts to open isomers over cyclic.

Keywords: open-cyclic tautomers; molecular switching; decay-associated spectra; warfarin, excited-state lifetime; cyclodextrins

Introduction

The fluorescence properties of coumarin derivatives[1] have attracted the attention of organic physical chemists for several decades. Our research group, in particular, synthesized two coumarin derivatives for fluorescent sensing of pH.[2] Like other fluorescent probes, coumarins display photophysical properties that are sensitive to their local environments, such as supramolecular host cavities.[3] Within this context, we have also exploited supramolecular host-guest approach alongside fluorescence behavior of a third coumain derivative, demonstrating a new sensing method for molecular recognition of optically inactive analytes.[4] During the course of our research, we realized the need to characterize the photophysical behaviors of warfarin (W) inside the cavities of cyclodextrins (CDs) macromolecular hosts (Scheme 1). W, which is one of the known fluorescent coumarin derivatives, is also a highly potent anticoagulant drug commonly prescribed by FDA as COUMADIN® for control and prevention of blood clots.[5]

There have been number of studies on the structures of W in different solvents and at different pH by NMR, UV–visible absorption and time-resolved fluorescence.[6-9] Early studies have concluded that the structure of drug in solution[6,7] at pH lower than its pK_a ~ 5 presents as either open or cyclic protonated form (predominantly as the structures in Scheme 1) and at pH higher than its pK_a, as deprotonated form (W^-), whose side chain is open. In addition to that, photophysical properties of W in different solvents and solvent mixtures were profoundly investigated.[8,9] Results confirmed that the protonated open form absorbs at λ = 310 nm, whereas the cyclic peak appears at λ = 280 nm. The results were then exploited to examine the binding of W to human serum albumin (HSA),[8] as well as model systems such as CDs.[9] In the later study, only steady-state fluorescence measurements have been undertaken, which warrants a more quantitative tool for examining the effects of supramolecular cavities. Accordingly, measurements of fluorescence lifetimes of W in the absence and presence of CDs at different pH are highly motivated.

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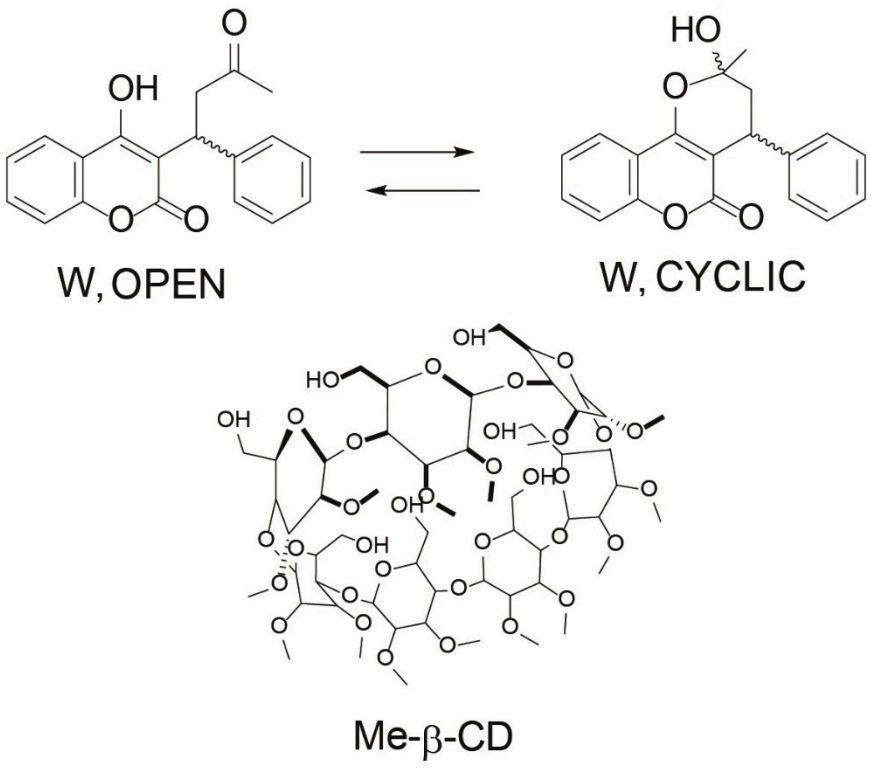
In this article, time-resolved fluorescence data of W have been collected as a function of pH and excitation wavelengths in water and inside *β*-CD molecular containers. We observed that excited-state lifetime of W increases upon binding to methyl-β-cyclodextrin (methyl-*β*-CD) (Scheme 1) and we were able to selectively monitor the interaction of each protonated tautomer (open vs cyclic) with the host molecules. We have specifically demonstrated while the length of excited-state lifetimes of the CD-encaged W^- depends on the effective viscosity of the surrounding microenvironment, extended fluorescence lifetime of the open protonated form of W upon enclathration is explained by the radiative-rate law. The difference in acidity between the two isomers of W is also investigated experimentally in the ground and excited state.



Scheme 1. Structural formulas of warfarin (W): open and cyclic isomers, and methyl-*β*-cyclodextrins (Me-*β*-CD) molecular container.

Experimental Section

Reagents. Wafarin (W), Acetyl-*β*-cyclodextrins (Ac-*β*-CD), Methyl-*β*-cyclodextrins (Me-*β*-CD), and (2-Hydroxyethyl)-*β*-cyclodextrins (HE-*β*-CD) were purchased from Sigma-Aldrich

Chemie GmbH, Taufkirchen, Germany (purity 99 %). Millipore water had conductivity less than 0.05 μS. The pH values of the solutions were adjusted (± 0.2 units) by adding adequate amounts of HCl or NaOH.

Absorption/steady-state fluorescence spectroscopy. The UV–visible absorption spectra were measured on Cary-300 instrument (Agilent) at room temperature, between 200 and 500 nm. Fluorescence spectra measurements were scanned at room temperature, between 300 and 550 nm on a Cary-Eclipse fluorimeter. Slit widths were 5 nm for both excitation and emission monochromators, unless otherwise specified. The pH values were recorded using a pH meter (WTW 330i equipped with a WTW SenTix Mic glass electrode). Quartz cuvettes (1.0 cm, 4.0 mL) were used in all spectroscopic measurements and were obtained from Starna Cells Inc. (Atascadero, California, USA).

pH-titration studies. The pH titration by UV–visible absorption spectroscopic method was accomplished by measuring the pH values of about 3 ml-solution in a rectangular quartz cuvette with 1-cm optical path length, and the absorption spectra were then recorded. To adjust pH, microliter volumes from 0.01 and 0.1 M NaOH and HCl solutions were pipetted consecutively to achieve the indicated pH values. The pK_a value was determined finally from fitting the titration data at a selected wavelength to a sigmoidal formula derived from Henderson-Hasselbalch and Beer-Lambert laws. The fitting algorithm was provided by SigmaPlot's software (version 6.1; SPCC, Inc., Chicago, Illinois, USA).

Steady-state binding titration studies. In the titration experiment, the total concentrations of the W were kept constant and that of the host was gradually increased. The pH of a certain volume of H_2O was first adjusted to either ~3 or ~9 in which a stock solution of W was prepared to give a final concentration of ~25 μM. A calculated weight of *β*-CD derivatives was added to the same solution of W to prepare the stock solution of the complex (~3.5 mM). The solutions with the final concentrations of *β*-CD were prepared by gradually adding

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increment volumes of the complex's stock solution to 2.4 ml of the free W directly in the quartz cuvettes. The absorption or fluorescence spectra were measured for each solution. The signals at certain wavelength were plotted as a function of host's total concentrations. The intermolecular interaction between *β*-CD and W may be quantified by the affinity constant referred to as the association equilibrium (*K*):

W + *β*-CD $\rightleftharpoons$ W-*β*-CD

$$K = \frac{[W-\beta-\mathrm{CD}]}{[W][\beta-\mathrm{CD}]} \quad (1)$$

$$C_{WF} = [W] + [W\text{-}\beta\text{-CD}] \quad (2)$$

$$C_{\beta\text{-CD}} = [\beta\text{-CD}] + [W\text{-}\beta\text{-CD}] \quad (3)$$

, where C_W and $C_{\beta\text{-CD}}$ mean the total concentrations of W and *β*-CD, respectively. It can be written that:

Y (Reading at certain *λ*) = constant 1 * [W] + constant 2 * [W-*β*-CD] (4)

Using Eqs.(1)-(4), we obtain

$$\Delta Y = \frac{\Delta(\mathrm{constant})C_{\beta-\mathrm{CD}}}{\frac{2}{KC_W-1-KC_{\beta-\mathrm{CD}}+\sqrt{(1-KC_W+KC_{\beta-\mathrm{CD}})^2+4KC_W}}+1} \quad (5)$$

,where ΔY = optical changes at a given λ; Δ(constant) = the difference between the molar absortivity of free and *β*-CD-complexed W in the case of absorption titration, and *K* = binding constant. The binding constants (*K*) were then evaluated by using the nonlinear formula of Eq. (5). Constant 2 was left as a floating parameter in the analysis by Levenberg-Marquardt algorithm, which was provided by SigmaPlot's software (version 6.1; SPCC, Inc., Chicago, Illinois, USA).

Time-resolved fluorescence measurements. The emission decays of W in the absence and presence of Me-*β*-CD at different pH values were collected using LifeSpec II spectrometer

that is based on TCSPC method with excitation at 280 and 320 nm using two Edinburgh laser diodes with repetition rate at 20 MHz and time resolution of ~90 picoseconds (ps). A red-sensitive high-speed PMT detector (Hamamatsu, H5773-04) and a colour filter (Edinburgh, standard set) with cut-off wavelength of 330 nm were used. Emission decays were collected every 10 nm over the entire emission spectra of W and W-β-CD complex in aqueous solution with a dwell time of 50 s at each wavelength. The data were globally fitted with mono-exponential and bi-exponential model functions depending on the tested sample, then convoluted with instrument response function (IRF) of ~90 ps. The time-resolved data were specifically analyzed using Edinburgh FAST software (Figure S1-S6, Supporting Information) in which decay-associated spectra (DAS) were constructed from the extracted intensity-contribution fraction (f_i) calculated from the pre-exponential amplitudes (B_i), as follows:

$$I(t) = \sum_i B_i exp(-t/\tau_i) \quad (6)$$

$$f_i = \frac{B_i \tau_i}{\sum_j B_j \tau_j} \quad (7)$$

Target analysis utilizing Glotaran software[11] were performed to confirm the kinetic expression for the population transfer of the two excited states that belong to free and CD-complexed W. Similar results were obtained by target analysis to those obtained by FAST (data not shown). The final results revealed that the two excited states decay mono-exponentially in parallel, which validate the interpretation of DAS in our work as described below.

Results and Discussion

Interactions of warfarin with cyclodextrins. Precedent studies on the interactions of W with several derivatives of CDs[9,12-14] have been taken into consideration while planning the experiments in this part. Accordingly, new derivatives of β-CD were selected, namely Ac-β-

CD and HE-*β*-CD (Chart S1, Supporting Information). The rationale behind our selection arises from the presence of additional hydroxyl or carbonyl functional groups that could enhance interactions with W. Unfortunately, both macrocycles gave weak or no interactions with W as monitored by UV–visible absorption measurements at pH 3 and 9 (Figure S7, Supporting Information). However, the previously examined derivative Me-*β*-CD[12] gave the highest binding constants, as illustrated in Figure 1. The data in Figure 1 were not published before and it was necessary to present them here to preclude the lifetime measurements below. Two pH values were selected 3 and 9 at which W persists as protonated and deprotonated form (W^-), respectively (see pH-titration results below). In Figure 1A at pH 3, the open protonated form, not cyclic, moderately reacts with the host (K= 2,900 M^{-1}), whereas W^- binds weakly CD at high pH (Figure 1B) with binding constant of 420 M^{-1} in agreement with previous reports.[9,13,14] Low binding affinities of coumarin to CD systems are not surprising because the presence of hydroxyl group is known to disadvantage inclusion. Upon addition of Me-*β*-CD host molecules (up to 40 equivalents) to the aqueous solutions of W at pH 3 and 9 (Figure 1), characteristic changes in the UV–visible absorption spectra were observed with the occurrence of several isosbestic points (311 nm, 315 nm, and 320 nm) confirming the formation of a 1:1 binding stoichiometry. The corresponding binding constants between W and Me-*β*-CD at a given pH were derived directly from the optical titration plot at a given wavelength using the formula described in the experimental section.

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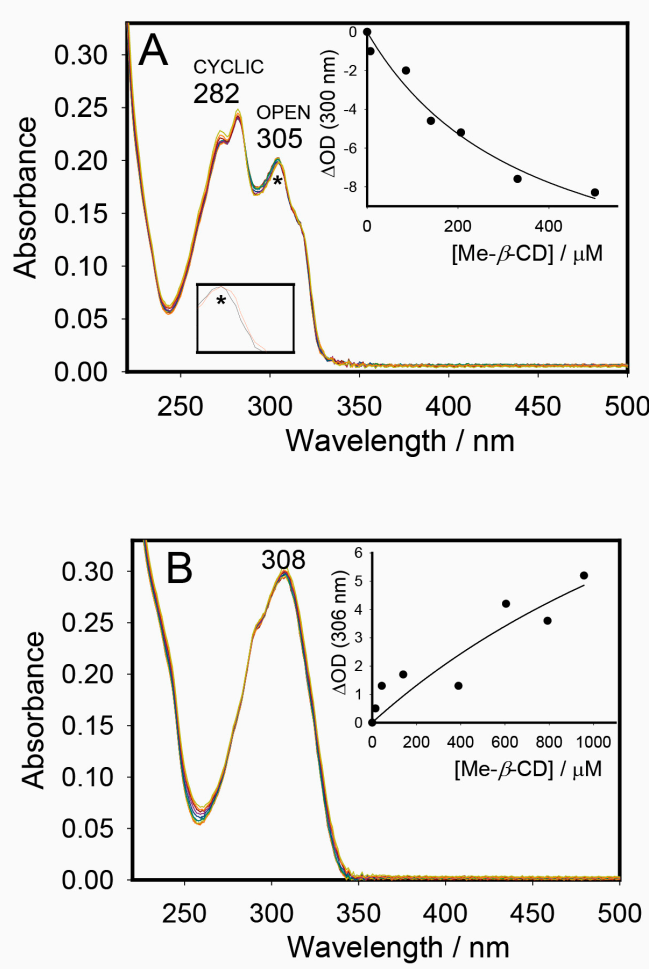

Figure 1. UV–visible absorption titration of W (25 μM) with Me-*β*-CD at pH 3 (A), and pH 9 (B); the *inset* shows the corresponding titration curve and the 1:1 binding fit (solid line) with $K = (2.9 \pm 0.3) \times 10^3$ M^{-1}, and $K = (4.2 \pm 1.0) \times 10^2$ M^{-1}, respectively.

Earlier report in octanol/water model system at pH 7.4 concluded that Cytochrome P450 2C9 (CYP2C9) has stabilized the cyclic form of W.[15]Absorption spectra upon addition of different amounts of *β*-CD to W in water at pH 7.4 (phosphate buffer) were later measured by Vasquez et al.[9] Authors concluded that W exists predominantly in its open form when bound to *β*-CD. They attributed this observation to a steric factor that forces W to remain in its open structure. Our findings at pH 3 support selective interactions of CD with the open form, during which only the longest absorption wavelength at ~305 nm of the engulfed protonated W in *β*-CD was affected (red shift from 305 to 306 nm), probably for similar steric reason to that suggested by Vasquez et al.

Optical measurements and host-induced pK_a shifts. A very recent experimental and theoretical investigations by Nowak et al.[16] on several hydroxycouamrin derivatives concluded that different location of hydroxyl group should affect the value of pK_a.[16] Motivated by this work, we looked at the changes of UV–visible absorption spectral profiles of free and CD-bound W in aqueous solutions as a function of pH values (Figure S8, Supporting Information). Me-*β*-CD-induced pK_a shifts corresponding to the deprotonation processes of the hydroxyl group in coumarin ring (Scheme 1) have already been studied before by Nowak et al.[12] yet our inspiration here specifically comes from the expected dependence of the extracted pK_a values on the selected wavelength in the corresponding titration plots (see experimental section). Accordingly, repeating these experiments besides those correspond to the new *β*-CD derivatives should enforce our understanding whether changing the position of hydroxyl group in the open and cyclic isomers would affect their pK_a values or not. Figure 2 illustrated the extracted pH titration plots for the cyclic (280 nm) and open (305 nm) W forms from data in Figure S8, Supporting Information. Positive shifts were observed upon addition of all CD derivatives in aqueous solution under similar conditions of ionic strength effects (see experimental section).[17] Regardless of type of isomer, Me-*β*-CD-assisted pK_a shifts are always larger than those induced by Ac-*β*-CD (ΔpK_a ~0.9 vs ~0.7 for open forms and ~0.6 vs ~0.3 for cyclic forms).The HE-*β*-CD induced the least pK_a shifts (ΔpK_a ~0.4 for open forms, and no shift observed in case of cyclic).

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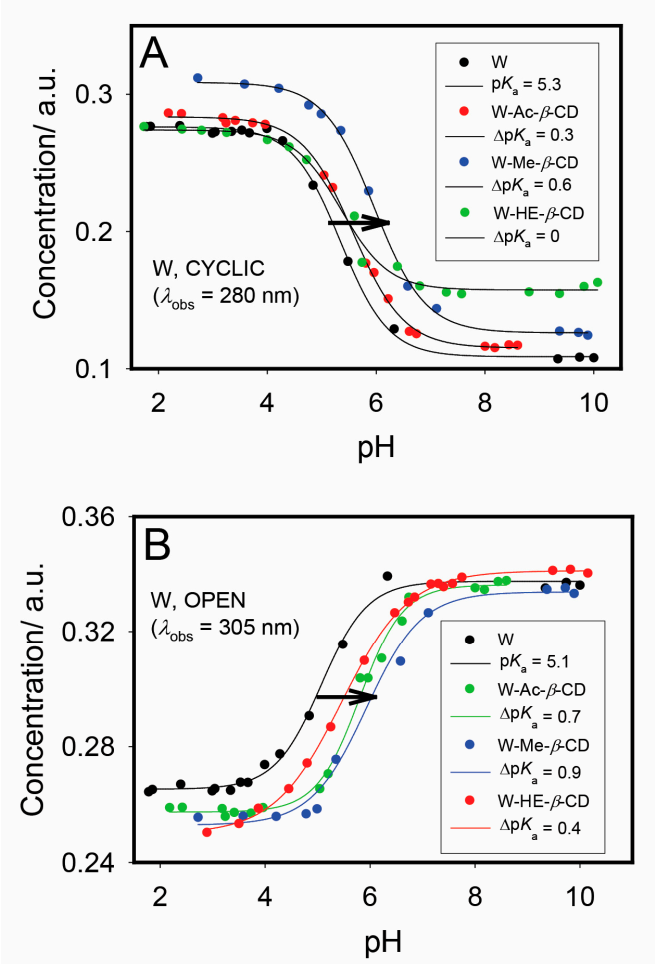

Figure 2. UV–visible absorption spectra of W in aqueous solutions at different pH values from 2-10 in water and inside the cavities of HE-*β*-CD, Ac-*β*-CD, and Me-*β*-CD. The experimental fitting error for the labeled pK_a values and the corresponding spectra are shown in Figure S8, Supporting Information.

The host-induced pK_a shifts reflect the changes in the binding affinities of drug to *β*-CD derivatives in the ground states (see Me-*β*-CD as an example at pH 3 and 9 in Figure 1), thus rationalized by preferential interactions of host towards the protonated form over deprotonated.[18] Nowak et al.[12] attributed the strongest pK_a shifts in Me-*β*-CD to the presence of methyl group that may have preferentially interacted with the CD cavity. More important to the focus of our paper is the observation that sequestration of drug into the three *β*-CD hosts increased pK_a of the open isomer more than that of cyclic, supporting the selective complexation with open form as concluded above. In addition to that, open form of free W

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appears to be more acidic in ground state (pK_a 5.1 vs 5.3), presumably due to the extended electron delocalization in the final charged product (W^-).[16]

Excitation, pH, and cyclodextrin dependence of warfarin steady-state fluorescence. Our paper specifically aimed at investigating the dependence of fluorescence of W on pH and excitation wavelength in the absence and presence of Me-*β*-CD, the host which has sufficiently interacted with W by virtue of the above absorption measurements. Fluorescence pH titration experiment was performed as illustrated in Figure 3. Different pK_a values were extracted upon exciting W in water at 280 and 320 nm (pK_a 6.1 vs 5.7), which is attributed to the deprotonation of the hydroxyl proton (Scheme 1), with the open form being more acidic over cyclic.

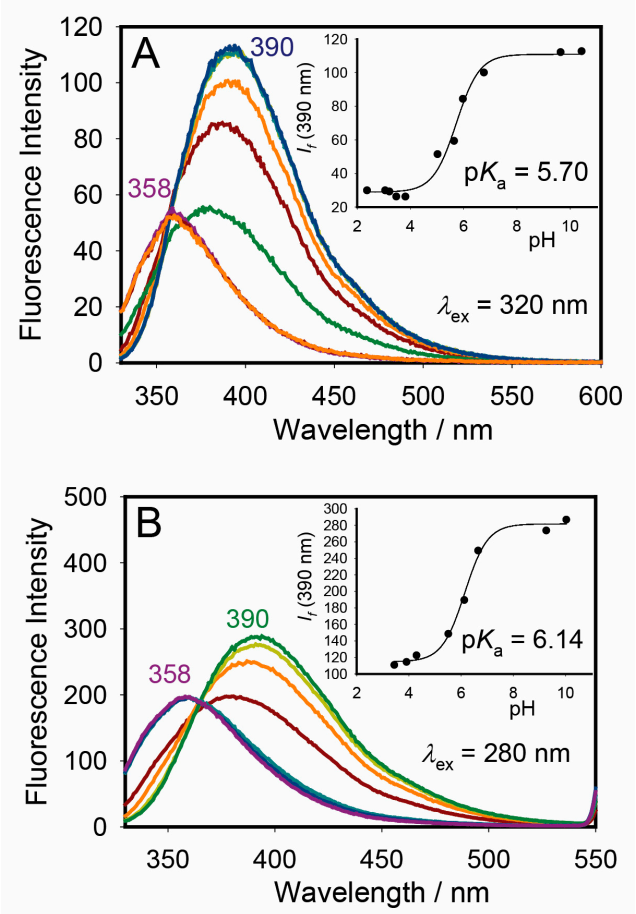

Figure 3. Fluorescence spectra of W (25 μM) at different pH values with excitation at 320 nm (A); and 280 nm (B); the *inset* shows the experimental fit to a sigmoidal function (solid line), which gives pK_a = 5.70 ± 0.07, and 6.14 ± 0.05, respectively. Slit widths were 5 nm for excitation and 10 nm for emission monochromators in spectra B.

This could be ascribed to extended electron delocalization in the excited-state structure of the corresponding deprotonated form in parallel to the behaviors of isomers in the ground-state.[16] It must be noted that open and cyclic forms, despite having similar emission maxima gave different emission profile when excited at 280 nm instead of 320 nm (Figure S9, Supporting Information), confirming the persistence of intramolecular proton transfer from the open form to cyclic tautomer (Scheme 1) in the excited state.

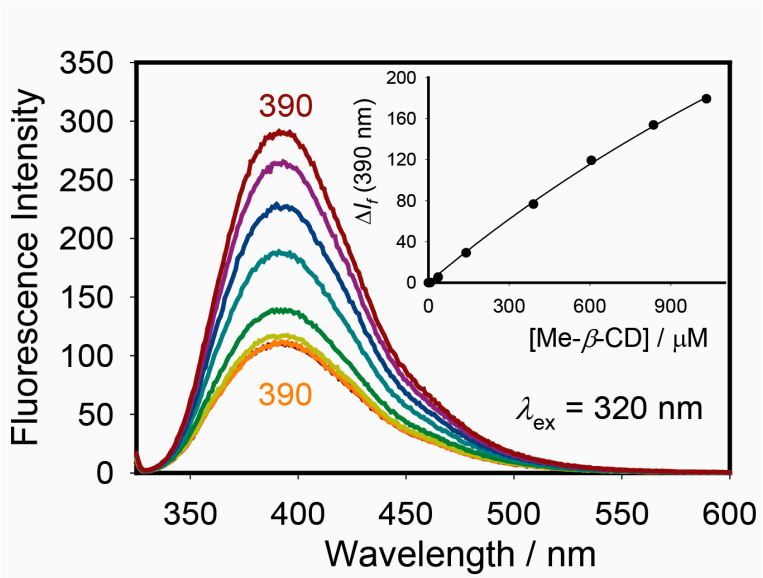

Figure 4. Fluorescence titration of W (25 μM) with Me-*β*-CD at pH 9 with excitation at 320 nm the *inset* shows the corresponding titration curve and the 1:1 binding fit (solid line) with $K = (2.66 \pm 0.06) \times 10^2$ M^{-1}.

As far as the effects of CDs on emission of W, we observed three-fold fluorescence enhancement of the encaged W^- by Me-*β*-CD at pH 9 (Figure 4; excitation at 320 nm) with binding affinity of 266 M^{-1} in agreement previous reports.[9,13,14] However, the protonated (open or cyclic) forms at pH 3 showed very weak enhancement in fluorescence upon addition of 10 equivalents of host (Figure S10, Supporting Information). Such contradictable results warrant further investigations using time-resolved fluorescence spectroscopy.

Fluorescence lifetime measurements/decay-associated spectra (DAS). We resorted to time-resolved fluorescence measurements to rationalize the lack of fluorescence enhancement of protonated W upon incorporation in Me-*β*-CD. We also sought to separate the complexation effects of host molecules on each protonated isomer (open vs cyclic) by monitoring the change in excited-state lifetimes of W upon selective excitation of each form at 320 and 280 nm. In previous reports in organic solvents, the two forms were simultaneously excited at 300 nm.[8] Emission decays collected every 10 nm across the entire emission spectra of free W at pH 3 upon excitation at 320 and 280 were globally fitted to monoexponential decay function, as shown in Figure S1 and S3 in the Supporting Information and tabulated in Table 1. The excited-state lifetimes of free W appear within our instrument resolution of ~90 ps in agreement with similar reports.[8,19] However, when Me-*β*-CD complexes were excited at pH 3 both monoexponential and biexponential decays were observed (Figure S4 and S6, Supporting Information). Although addition of CD at pH 3 did not affect the position of peaks (390 nm), excited-state lifetime increased but only when the complex was excited at 320 nm, not 280 (Figure 5, S4 and S6, Supporting Information), supporting that Me-*β*-CD host favors the open tautomer form. Even though we collected emission decays of drugs in Figure 5 upon addition of 40 equivalents of host (as limited by its solubility in water of about 10 mM), complete formation of complex has not been achieved due to relatively weak binding constants.

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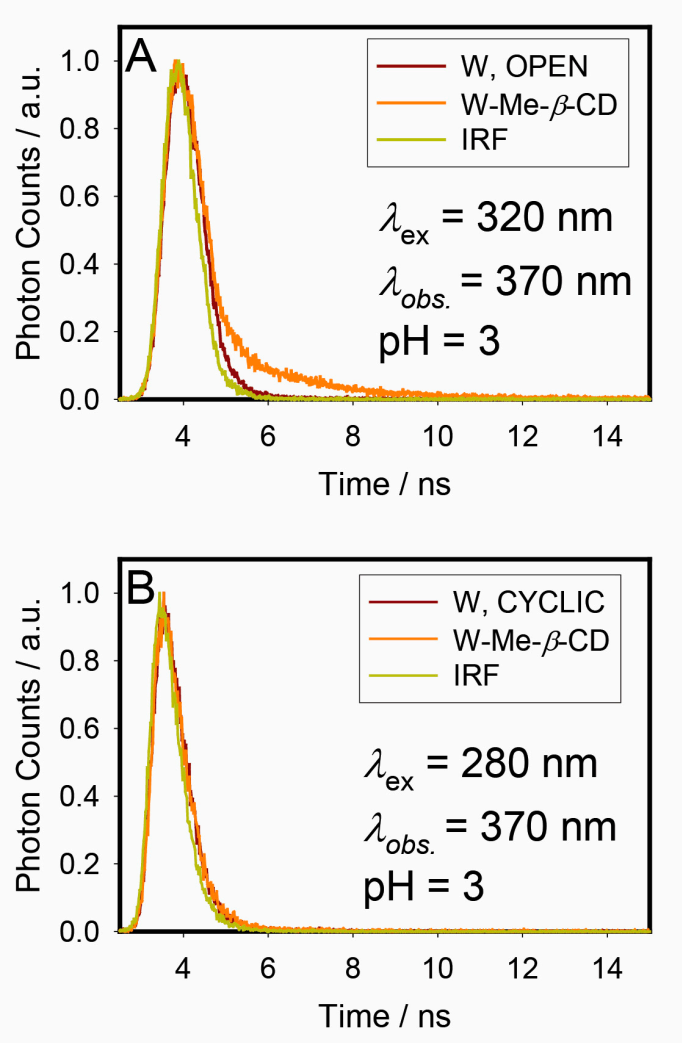

Figure 5. Emission decays collected at 370 nm for W at 25 μM in the absence and presence of Me-β-CD (1.0 mM) upon exciting at 280 nm (A) and 320 nm (B). No change in fluorescence lifetime upon excitation at 280 nm. IRF is the instrument response function of ~ 90 ps.

Accordingly, the corresponding DAS spectra in Figure 6 (see experimental section) are best interpreted by assuming contribution from both free and CD-complexed drug. The emission of free drug at pH 3 dominates the emission spectrum with emission band centered at ~358 nm (Figure 6A). The corresponding complex at this pH has an emission peak at ~375 nm with a longer excited-state lifetime of ~ 2.43 ns, yet much lower emission quantum yields (0.006 vs 0.0005 in Table 2).

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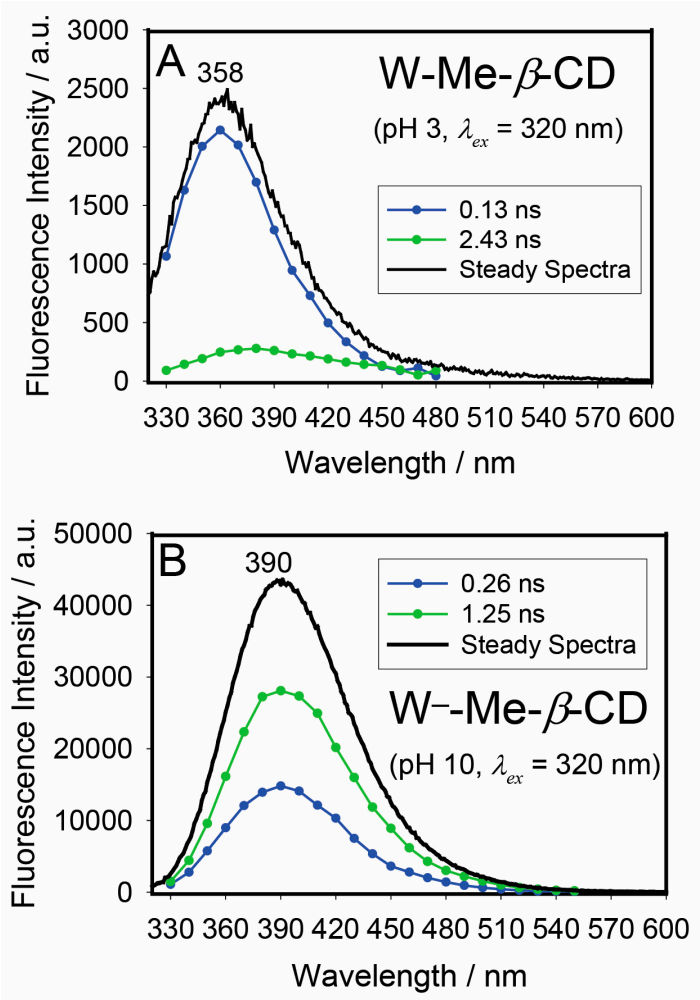

Figure 6. Decay-associated spectra (DAS) of two-component mixture of fluorophores for W-Me-*β*-CD host-guest complex (25 μM for W, and 1 mM for W-Me-*β*-CD) at pH 3 and 9 and upon excitation at 320 nm and room temperature. The corresponding steady-state spectra of each solution are also shown for comparison (see experimental section).

Association of each extracted lifetime to each species by DAS method enables us to track the changes in the corresponding radiative (k_r) and non-radiative rate (k_{nr}) constants alongside emission quantum yields (Φ) upon complexation of W to Me-*β*-CD, as illustrated in Table 2. The variations of k_r values in the absence and presence of CD are difficult to discuss, because of the unreliable measurement of lifetimes in water. However, the red shifts of emission peaks of W upon complexation to Me-*β*-CD hosts at pH 3 despite their non-polar hydrophobic and rigid cavity along with concomitant significant decrease of the k_r values by ~2 orders of magnitude, from $k_r = 4.6 \times 10^7$ to 2×10^5 s^{-1}, means that the enhancement in excited-state

lifetimes is best described by radiative-rate law.[10] This argument is supported by the boarder UV–visible spectrum of W when compared to that spectrum in water upon inclusion to CD, as shown in Figure 1A and in light of Strickler-Berg equation.[10] Karlsson et al.[8] pointed out the possibility of attributing the longer lifetime of W observed in ethanol (0.45 ns, λ_{ex} = 295 nm) to the formation of solute-solvent hydrogen-bonded complexes. Dondon et al.[19] posited that for other 4-Hydroxycoumarin derivatives similar hydrogen-bonded complexes could have formed between the coumarin lactone group and the CD secondary hydroxyl groups. It transpires that there is a plausible explanation to the fluorescence behaviors of protonated W-CD systems that similar hydrogen-bonded complexes have formed between CD secondary hydroxyl group and the carbonyl group of open form, which the cyclic form lacks.

Emission decays collected at pH 9 (Figure S2 and S5, Supporting Information) gave DAS spectra (Figure 6B) that demonstrated opposite effects induced by the addition of macromolecular host. In agreement with previous reports,[9,13,14] the complex at pH 9 excited at 320 nm has higher emission yield than that of free drug with excited-state lifetime of ~1.25 ns and no change in peak position at ~ 390 nm (Table 2). Vasquez et al. suggested encapsulation of deprotonated W^- forms by Me-β-CD considerably suppresses the vibronic modes that provide pathways for non-radiative transitions between the excited and ground states of W, causing a decrease in the rates of the non-radiative decay processes. Indeed, our calculation supports these findings within the context of energy-gap law. The k_{nr} values of W in Table 2 decreased ~5 times upon inclusion in the hydrophobic interior of Me-β-CD, whereas k_r values decrease only ~2 times. The increase in lifetimes and associated decrease in k_{nr} of other 4-hydroxycoumarin derivatives[19] from below 0.5 ns to 1.1-1.3 ns (depending on the derivative) were also observed upon sequestration of dyes into β-CD macromolecules.

Table 1. Spectroscopic and photophysical data of W at 25 µM in the absence and presence of Me-*β*-CD (1.0 mM). The DAS maximum for each lifetime is given in bracket.

Drug Forms	λ_{abs}/ nm	ϵ/ $M^{-1}cm^{-}$ 1	λ_{ex}/ nm	λ_{em}/ nm	τ_1/ ns (λ_{em}/ nm)	τ_2/ ns (λ_{em}/ nm)
Free (pH 3)	305	10 680	320	358	~ 0.18	
	282	12 820	280	358	~ 0.21	
Free (pH 9)	309	13 532	320	390	~ 0.11	
Complex* (pH 3)	306	10 102	320	358	~ 0.13 (358)	2.43 (375)
Complex (pH 9)	309	13 580	320	390	~ 0.26 (390)	1.25 (390)

* With excitation at 280 nm, no change in excited-state lifetime was observed.

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Table 2. Fluorescence quantum yield Φ_F, radiative rate constant k_r, and non-radiative rate constant k_{nr} of different W species.

Drug Forms	λ_{ex}/ nm	Φ_F ($\times 10^{-3}$)[a]	k_r ($\times 10^7$ s^{-1})[b]	k_{nr} ($\times 10^9$ s^{-1})[b]	τ/ ns
W OPEN	320	6 ± 0.01	~ 4.6 ± 0.01	~ 7.6 ± 0.01	~ 0.13
W^-	320	12 ± 0.01	~ 4.6 ± 0.01	~ 3.8 ± 0.01	~ 0.26
W-Me-β-CD OPEN	320	0.5 ± 0.01	0.02 ± 0.01	0.4 ± 0.01	2.43
W^--Me-β-CD	320	24 ± 0.01	1.9 ± 0.01	0.8 ± 0.01	1.25

[a]Measured using W in phosphate-buffered saline (PBS, pH ~7.4, 10 mM sodium phosphate) as the standard (Φ_F = 0.012),[9] and calculated using the known equation:[10] $\emptyset_{unk} = \emptyset_{std}\left(\frac{I_{unk}}{A_{unk}}\right)\left(\frac{A_{std}}{I_{std}}\right)\left(\frac{n_{unk}}{n_{std}}\right)^2$, where n is the refractive indices for the standard (std) and experimental (unk) solvents, I is the fluorescence integral of the emission between 300 and 550 nm, and A is the absorbance at the excitation wavelength.

[b]Calculated using the known equations: $\emptyset = \frac{k_r}{k_r+k_{nr}}$, $\tau = \frac{1}{k_r+k_{nr}}$, $k_{nr} = \frac{1-\emptyset}{\tau}$, and $k_r = \frac{\emptyset}{\tau}$.

Conclusions

In this work, quantitative time-resolved fluorescence spectra of W measured using picoseconds laser diode with selective excitation of protonated isomers at 280 and 320 nm were conducted utilizing both global and target analysis to give more specific information about structures and kinetic behaviors of excited-states of protonated and deprotonated drugs in water and inside CDs. We observed an increase in excited-sate lifetime of open protonated form upon its selective binding to Me-*β*-CD, over cyclic. Lifetime of deprotonated form has also increased upon inclusion. The increase in lifetime of open protonated form was explained by radiative rate law, while that of deprotonated form by energy-gap law. We have also demonstrated the lower acidity of the open form. With the aid of absorption and time-resolved fluorescence spectroscopies, we postulate the selective formation of hydrogen-bonded complexes in both the ground and excited states between the open form and the host.

Fluorescence properties of W inside CDs in aqueous solutions at different pH values have attracted attentions in several occasions due to their implications for bioanalytical quantification of W in commercial pesticides[14,20,21] and biological liquids.[22] Recent studies also exploited W-CD system as fluorescent probes and site markers in drug-protein interactions.[23,24] In addition to fluorescence properties, better understanding of the interactions of W with CDs in ground and excited states at different pH values should lead, in the future, to better modulating drugs pK_a values and open-cyclic switchable properties in solution[12,13] and in different microheterogeneous environment that could advance their analytical separation by electrophoresis[17] and liquid chromatography,[25,26] as well as their formulations[27] and clinical/biomedical applications.[28] results should, therefore, lead to better understanding of the role of CD on manipulating the open-cyclic switchable structure/function of this anticoagulant drug in ground and excited states.

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Author Contributions

NA conducted all experiments. NA and NS prepared figures, analyzed and discussed the results. NS wrote the main manuscript, supervised the work, and edited the manuscript. Both authors reviewed the manuscript.

Additional Information

Supplementary information

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