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Dynamics of complexation of flavone and chromone to β-cyclodextrin

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Abstract

The complexation dynamics of the excited triplet states of flavone and chromone with β-cyclodextrin was studied by laser flash photolysis. The exit and entry rate constants for the triplet states of these ketones were determined using Cu^{2+} and NO_2^- as quenchers. The entry and exit rate constants depend on the size of the guest molecule and were faster for chromone than for flavone.

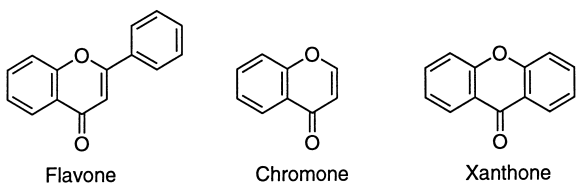
Keywords: Triplet state; Cyclodextrins; Flavone; Chromone; Absorption spectra

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides that provide a relatively hydrophobic cavity for the complexation of a variety of guest molecules [1–4]. The size of the CD cavity is determined by the number of glucose units in the molecule (6, 7 or 8 for α-, β- and γ-CD). CDs have been extensively used as host molecules to understand fundamental aspects of host–guest complexation, to enhance solubilization, to protect guest molecules from degradation and to provide building blocks for functional supramolecular systems. In addition, CDs were employed as enzyme mimetic systems [1,5–9].

The complexation efficiency of guests with CDs is determined by relatively weak interactions, such as van der Waals forces, dipole–dipole interactions and hydrogen bonds [4]. In addition, topological aspects such as size complementarity between the guest and CD cavity influence the complexation efficiency. In most cases, the characterization of CD complexes includes the determination of equilibrium constants and binding stoichiometries [2,10]. Much less information is available on the complexation dynamics of guests with CDs [11–20]. Kinetic information is valuable when CDs are employed to perform specific functions, such as catalysis or transport. Information on the complexation dynamics cannot be obtained from thermodynamic parameters, such as equilibrium constants.

In recent years, we have made a concerted effort to understand the parameters that affect the entry and exit processes of guests with CDs. Initial studies centered on xanthone [15–17] for which relocation can be followed directly due to the unique photophysics of its excited triplet state [21–23]. These studies were expanded to 1-naphthyl-1-ethanol and 2-naphthyl-1-ethanol as guest molecules [19]. The exit rate constants of the triplet states of both molecules from β-CD complexes with 1:1 (guest:CD) stoichiometries were at least one order of magnitude smaller than the exit rate constant observed for triplet xanthone. In this work, we chose to use flavone and chromone (Scheme 1) as guest molecules to obtain further information on how the structure of the guests affects the complexation dynamics. When compared to xanthone, flavone and chromone have different sizes, but all molecules have the 4H-pyran-4-one moiety.



Scheme 1.

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2. Experimental details

2.1. Materials

β-CD was a generous gift from Cerestar (lot F6080-191) and was used as received. Chromone (Aldrich, 99%), flavone (Aldrich, 99%) and xanthone (Aldrich, 97%) were recrystallized from ethanol/water. The purity of these ketones was checked by gas chromatography (Fissons GC 800) or HPLC (Hewlett Packard 1100). $NaNO_2$ (Aldrich, 97%) was recrystallized from ethanol/water. D_2O (Cambridge Isotope Laboratories, 99.9%), $CuSO_4$ (BDH) and N_2O (Praxair USP) were used as received. Deionized water (Sybron-Barnstead) was employed for all samples.

2.2. Instrumentation

Absorption spectra were obtained on Cary 1 or Cary 5 spectrophotometers at room temperature (20±2°C). ^{1}H NMR spectra were obtained on a 360 MHz spectrometer (Bruker AMX 360). Chemical shifts were measured with respect to the residual water signal at δ=4.65 ppm.

The laser flash photolysis system employed to measure the triplet–triplet absorption spectra and triplet decay kinetics was described previously [17]. Samples were excited at 308 nm with a Lumonics excimer laser (EX-510) or at 266 nm with a Spectra Physics YAG laser (GCR-12). All measurements were performed at 20±2°C. Flavone and chromone are easily photoionized in aqueous solutions. The laser energy was diminished using neutral density filters to minimize the formation of solvated electrons. Alternatively, samples were purged with N_2O, which is an efficient solvated electron trap [24]. Samples contained in 7 mm×7 mm Suprasil cells were purged for 20 min with either N_2 or N_2O.

2.3. Methods

Saturated flavone (≤0.12 mM) and xanthone (≤0.05 mM) solutions in water were prepared by stirring the solid in water for 24 h followed by filtration through 0.45 μm Millipore filters. Chromone is soluble in water and aqueous solutions were prepared (ca. 0.08 mM) to obtain an absorbance of ca. 0.3 (l=7 mm) at 308 nm. CD solutions at the highest CD concentration were prepared by dissolving the solid in the flavone, xanthone or chromone aqueous solutions. These solutions were left stirring for at least 4 h. Solutions at lower CD concentrations were prepared by dilution with the ketone aqueous solutions and were left stirring for 24 h. Samples for ^{1}H NMR were prepared in D_2O. Quencher solutions were prepared by dissolving the salts in aqueous solutions containing the ketone and CD.

3. Results

3.1. Ground-state complexation of flavone and chromone to β-CD

^{1}H NMR spectroscopy was employed to establish if flavone and chromone were incorporated into the β-CD cavity. Signals for the protons of chromone (10 mM) in D_2O were observed between 6.3 and 8.07 ppm. In the presence of 10 mM β-CD these signals shifted downfield by 0.01–0.07 ppm. In the case of flavone (0.12 mM) in D_2O the ^{1}H NMR signals were observed between 6.83 and 7.46 ppm. In the presence of 1 mM β-CD all signals for the flavone were shifted. In contrast to chromone, some signals shifted upfield whereas others shifted downfield. The absolute magnitude of these shifts varied between 0.03 and 0.07 ppm. These ^{1}H NMR results showed that both guest molecules were incorporated into β-CD cavities.

Upfield shifts for the ^{1}H NMR signals of CDs are indicative that aromatic guest molecules are located close to the protons for which a shift is observed [25]. The H_3 and H_5 protons of the glucose units are located inside the CD cavity, whereas protons H_6 are located at the rim with the primary alcohols and protons H_2 and H_4 are at the opposite entrance of the cavity. When chromone was added to β-CD solutions no shifts were observed for the β-CD signals corresponding to the H_2 and H_4 protons of the glucose units. For protons H_6, H_3 and H_5 an upfield shift between 0.03 and 0.11 ppm was detected, suggesting that chromone is incorporated within the CD cavity close to the entrance with the primary alcohols. In the case of flavone only small shifts (0.01 ppm) were observed for the H_5 and H_6 protons of β-CD. This results suggests that flavone is also incorporated into the CD cavity, but the reasons for the small magnitude of the shifts are not immediately apparent. The ^{1}H NMR spectra could not be employed for the determination of equilibrium constants and binding stoichiometries because of the poor signal-to-noise ratios in particular in the case of flavone.

The absorption spectra of flavone [26] and chromone [27] are dependent on the solvent polarity. A blue shift was observed for the absorption band at the lowest energy in isopropanol when compared to that in water. In the presence of increasing β-CD concentration the absorption spectra of flavone (Fig. 1) and chromone shifted to shorter wavelengths. This effect is more pronounced for flavone than for chromone.

The equilibrium constants (K_{eq}) between flavone or chromone and β-CD were obtained from the analysis of the change in the ground-state absorption of the ketones (ΔA) with increasing CD concentrations (Eq. (1)). This equation is valid when the concentration of CD is in excess over the guest concentration [28].

$$\Delta A = \frac{[G]_T \Delta_X K_{eq}[CD]}{1 + K_{eq}[CD]} \quad (1)$$

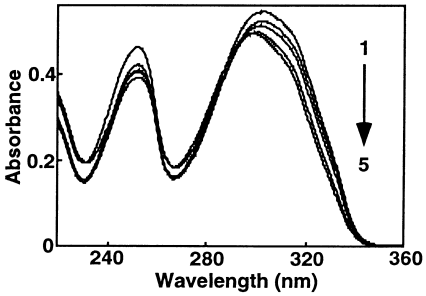

Fig. 1. Absorption spectra of ca. 30 μM flavone in water in the absence (1) and presence of (2) 0.1 mM, (3) 1.2 mM, (4) 3.0 mM and (5) 12 mM β-CD.

The total guest (flavone or chromone) concentration is given by $[G]_T$ and Δ_X corresponds to the difference in the extinction coefficient of the guest in water and when complexed to CD. This non-linear equation is preferred when determining the values for K_{eq} because it properly weights the experimental data.

A 1:1 complexation stoichiometry is assumed in Eq. (1). The validity of this assumption was checked by plotting the data in the form of a double-reciprocal plot (Benesi–Hildebrand plot) of Eq. (1) [29]. A linear relationship is expected if the correct stoichiometry is assumed.

$$\frac{1}{\Delta I} = \frac{1}{[G]_T \Delta_X} + \frac{1}{[G]_T \Delta_X K_{eq}[CD]} \quad (2)$$

Linear double-reciprocal plots were observed for flavone (inset Fig. 2) and chromone when the β-CD concentration was larger than 0.5 mM, indicating that for both guest molecules the complexation stoichiometry was 1:1 (guest:CD). The deviation from linearity for the lower CD concentrations was probably related to the fact that the measured differences in the absorbance values were small and for this reason the associated errors were large. The K_{eq} values determined for flavone (Fig. 2) and chromone from the fit of the experimental data to Eq. (1) were 1090±80 and 240±40 M^{-1}, respectively.

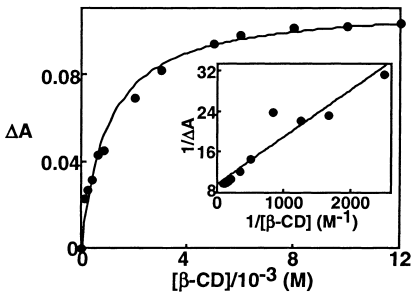

Fig. 2. Changes in the flavone (ca. 30 μM) absorption as a function of the CD concentration. The solid line corresponds to the fit of the experimental data to Eq. (1). The inset shows the double-reciprocal plot and the solid line corresponds to the fit to Eq. (2).

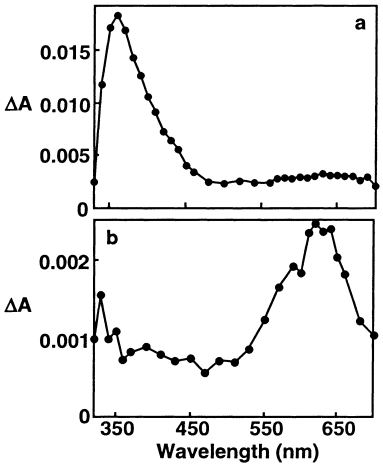

Fig. 3. Triplet–triplet absorption spectra for aqueous solutions of (a) flavone (27 μM at a delay of 7 μs) and (b) chromone (120 μM at a delay of 5 μs).

3.2. Dynamics of excited triplet state complexation to CD

The triplet–triplet absorption spectra of flavone and chromone in water showed a relative intense absorption band above 600 nm (Fig. 3). The spectra for flavone and chromone in water were similar to those previously reported in acetonitrile [26,30,31]. For both ketones we observed an additional absorption above 600 nm at short delays after the laser pulse. This absorption disappeared when N_2O was added to the solution, suggesting that it is due to solvated electrons formed in the photoionization of the ketones. This process has been shown to be efficient in water and its efficiency can be enhanced in the presence of organized systems such as micelles and CDs [32,33]. To avoid the interference of the absorption due to solvated electrons the laser power was decreased or N_2O was added to the solution. The presence of N_2O did not affect the flavone or chromone triplet lifetimes in the absence or presence of CD. The addition of N_2O had the advantage that it did not lead to a decrease of the triplet absorption signal as was observed when the laser power was decreased. In the absence of solvated electrons the decay kinetics measured at 630 nm follows a mono-exponential function and the triplet lifetime for flavone in water was longer than 15 μs. This lifetime is somewhat dependent on the presence of residual oxygen that quenches the excited triplet state. The triplet lifetime of chromone in water was longer than 1 μs but it was very dependent on the chromone ground state concentration, since self-quenching of the triplet is very efficient [31]. In the presence of β-CD no shifts were observed for the triplet–triplet absorption spectra of flavone and chromone when compared to those observed in water. However, the triplet lifetimes were somewhat longer than observed for the same experimental conditions in water.

The quenching methodology was employed to obtain the values for the entry (k_+) and exit (k_-) rate constants of the

Table 1
Quenching rate constants for xanthone, flavone and chromone in water, quenching, exit and entry rate constants for guests complexed to β-CD (5 and 10 mM)[a]

Guest	Quencher	k_{qo} ($M^{-1}\,s^{-1}$)	k_{qH} ($M^{-1}\,s^{-1}$)	k_+ ($10^9\,M^{-1}\,s^{-1}$)	k_- ($10^7\,s^{-1}$)
Xanthone	Cu^{2+}	$(5.6\pm0.4)\times10^7$	$0–2\times10^6$	1.1±0.1	1.2±0.1
	No quencher			0.4±0.1[b]	0.84±0.07[b]
Chromone	Cu^{2+}	$(8.6\pm0.4)\times10^7$	$0–1\times10^6$	3±1	2.1±0.1
	NO_2^-	$(5.3\pm0.2)\times10^9$	$0–1\times10^7$	7±3	5.5±1.0
Flavone	Cu^{2+}	$(4.5\pm0.3)\times10^7$	$0–1\times10^6$	2.4±1.2	0.44±0.18
	NO_2^-	$(4.0\pm0.1)\times10^9$	$0–1\times10^9$	2–20	1–7
1-Naphthyl-1-ethanol[c]	Mn^{2+}	$(2.6\pm0.4)\times10^7$	4.3×10^6	0.47±0.19	0.048±0.018
2-Naphthyl-1-ethanol[c]	Mn^{2+}	$(2.6\pm0.4)\times10^7$	2.5×10^6	0.29±0.16	0.018±0.007

[a] The errors for k_{qo} correspond to the statistical errors for the fit of the data to Eq. (3), the ranges for k_{qH} correspond to those for which the fit of the data to Eq. (4) is acceptable and the errors for k_+ and k_- correspond to the average deviation for the maximum and minimum values recovered when k_{qH} was fixed to the range quoted.
[b] From [16].
[c] From [19].

triplet ketones with β-CD. Details on this methodology were previously described [20,34], and it has been successfully applied to determine the complexation dynamics of guest molecules with CDs [12–14,19]. Briefly, a quencher is used that primarily resides in the aqueous phase and for this reason it has a higher quenching efficiency for the guest in water (k_{qo}) than when the guest is complexed to the CD cavity (k_{qH}).

The values for k_{qo} were determined from quenching plots in water in the absence of CD. The observed triplet decay rate constants (k_{obs}) was determined at several quencher concentrations and the data were fitted to Eq. (3), where k_o was the ketone decay rate constant in water in the absence of quencher.

$$k_{obs} = k_o + k_{qo}[Q] \quad (3)$$

The plots for the quenching of triplet flavone and chromone in water by NO_2^- or Cu^{2+}, as well as the plots for xanthone quenched by Cu^{2+} were linear. The quenching of triplet flavone or chromone by NO_2^- was five to ten times more efficient than the quenching by Cu^{2+} (Table 1).

The quenching plots were curved when the excited triplet ketones were quenched by Cu^{2+} in the presence of β-CD (Fig. 4). This result suggests that the quenching of the triplet states inside the CD cavity was less efficient than in water and that the complexation dynamics occurs on the same time scale as the quenching processes. In the presence of nitrous oxide the triplet decays were mono-exponential at all quencher concentrations employed. The dependence of the observed rate constant with the quencher concentration is given by Eq. (4), where k_o and k_H are the triplet lifetimes in water and in the presence of CD, and k_+ and k_- are the entry and exit rate constants for the triplet ketones.

$$k_{obs} = k_H + k_- + k_{qH}[Q] - \frac{k_- k_+ [CD]}{k_o + k_{qo}[Q] + k_+[CD]} \quad (4)$$

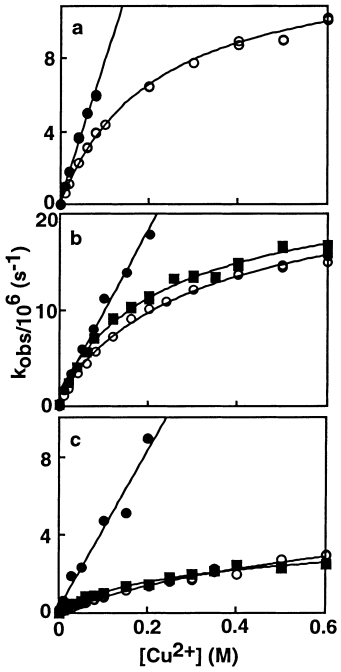

Fig. 4. Quenching plots with Cu^{2+} for the triplet excited states of (a) xanthone, (b) chromone and (c) flavone in (●) water and in the presence of (○) 5 mM β-CD or (■) 10 mM β-CD. Only a small portion of the quenching plot in water is shown for clarity. The solid line for the quenching plot in water corresponds to the fit to Eq. (3). In the presence of β-CD the experimental data were fitted to Eq. (4). (a) $k_o=5\times10^4\,s^{-1}$, $k_H=4.3\times10^4\,s^{-1}$, $k_{qo}=5.6\times10^7\,M^{-1}\,s^{-1}$, $k_{qH}=2.1\times10^6\,M^{-1}\,s^{-1}$, $k_+=9.5\times10^8\,M^{-1}\,s^{-1}$ and $k_-=1.12\times10^7\,s^{-1}$; (b) $k_o=1.3\times10^6\,s^{-1}$, $k_{qo}=8.6\times10^7\,M^{-1}\,s^{-1}$, $k_{qH}=1\times10^6\,M^{-1}\,s^{-1}$, for [β-CD]=5 mM: $k_H=2.37\times10^5\,s^{-1}$, $k_+=3.7\times10^9\,M^{-1}\,s^{-1}$ and $k_-=2.2\times10^7\,s^{-1}$ and for [β-CD]=10 mM: $k_H=2.23\times10^5\,s^{-1}$, $k_+=2.5\times10^9\,M^{-1}\,s^{-1}$ and $k_-=2.2\times10^7\,s^{-1}$. (c) $k_o=4\times10^5\,s^{-1}$, $k_{qo}=4.5\times10^7\,M^{-1}\,s^{-1}$, $k_{qH}=1\times10^6\,M^{-1}\,s^{-1}$, for [β-CD]=5 mM: $k_H=2.95\times10^4\,s^{-1}$, $k_+=1.6\times10^9\,M^{-1}\,s^{-1}$ and $k_-=2.6\times10^6\,s^{-1}$ and for [β-CD]=10 mM: $k_H=2.94\times10^4\,s^{-1}$, $k_+=2.5\times10^9\,M^{-1}\,s^{-1}$ and $k_-=4.4\times10^6\,s^{-1}$.

The values for k_o and k_{qo} were determined from studies in the absence of CD. The k_H values correspond to the decay rate constant of the triplet ketone in the presence of β-CD, but absence of quencher. When the experimental data were fitted to Eq. (4) adequate plots were observed for a wide range of values for k_-, k_{qH}, and k_+. This is a consequence of the fact that at the highest quencher concentration employed the quenching plots did not approach the constant value (k_{qH}=0) or the linear relationship (k_{qH}>0) expected from Eq. (4) at high quencher concentrations. The lowest possible value for k_{qH} is 0, which corresponds to complete protection of the complexed triplet states. Several fits were performed by fixing k_{qH} to 0 and incrementally larger values until the fit to the experimental data was not adequate. The values for k_+ and k_- were recovered for the range of k_{qH} values that led to adequate fits. The entry and exit rate constants in Table 1 correspond to the average of the minimum and maximum values recovered from adequate fits.

We also used the quenching methodology to determine the exit and entry rate constants of triplet xanthone with β-CD (Fig. 4, Table 1). The dynamics of xanthone had been previously studied using the direct methodology [15,16,18]. The decay for triplet xanthone was measured at 600 nm, which corresponds to the isosbestic point of the triplet absorption for xanthone in water and when complexed to β-CD [15]. The decays for the triplet xanthone at 600 nm in the presence of β-CD and quencher were mono-exponential and the variation of k_{obs} with the Cu^{2+} concentration was fitted to Eq. (4) (Table 1).

The complexation dynamics of triplet flavone and chromone was also studied using nitrite as a quencher. Curved quenching plots were observed for both ketones in the presence of β-CD (Fig. 5). In the case of chromone the quenching plot was fitted in the same manner as described for the quenching with Cu^{2+} and somewhat higher values for k_+ and k_- were recovered when nitrite was used as a quencher (Table 1). However, in the case of flavone much less curvature was observed in the quenching plots and adequate fits were observed for a very wide range of k_{qH}. In addition, the upper limit for the recovered entry rate constants does not have physical significance since k_+ cannot be higher than the diffusional rate constant in water ($6.5\times10^9\,M^{-1}\,s^{-1}$) [35].

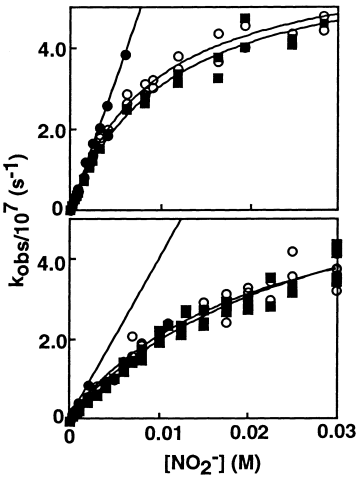

Fig. 5. Quenching plots with NO_2^- for the triplet excited states of (a) chromone and (b) flavone in (●) water, (○) 5 mM β-CD and (■) 10 mM β-CD. Only a small portion of the quenching plot in water is shown for clarity. The solid line for the quenching plot is water corresponds to the fit to Eq. (3). In the presence of β-CD the experimental data were fitted to Eq. (4). (a) $k_o=6\times10^5\,s^{-1}$, $k_{qo}=5.3\times10^9\,M^{-1}\,s^{-1}$, $k_{qH}=1\times10^7\,M^{-1}\,s^{-1}$, for [β-CD]=5 mM: $k_H=2.37\times10^5\,s^{-1}$, $k_+=9.6\times10^9\,M^{-1}\,s^{-1}$ and $k_-=6.3\times10^7\,s^{-1}$ and for [β-CD]=10 mM: $k_H=2.23\times10^5\,s^{-1}$, $k_+=5.7\times10^9\,M^{-1}\,s^{-1}$ and $k_-=6.3\times10^7\,s^{-1}$. (b) $k_o=4\times10^5\,s^{-1}$, $k_{qo}=4.0\times10^9\,M^{-1}\,s^{-1}$, $k_{qH}=1\times10^7\,M^{-1}\,s^{-1}$, for [β-CD]=5 mM: $k_H=2.95\times10^4\,s^{-1}$, $k_+=1.7\times10^{10}\,M^{-1}\,s^{-1}$ and $k_-=6.3\times10^7\,s^{-1}$ and for [β-CD]=10 mM: $k_H=2.49\times10^4\,s^{-1}$, $k_+=1.1\times10^{10}\,M^{-1}\,s^{-1}$ and $k_-=7.1\times10^7\,s^{-1}$.

4. Discussion

The binding efficiency of guests with similar functionalities to CD cavities is primarily determined by the size of the guest molecule. In the case of xanthone, it was shown that optimum binding occurred with β-CD, whereas the cavities of α-CD and γ-CD were respectively too small and too large for xanthone complexation. As a consequence the equilibrium constants for xanthone with CDs was highest for β-CD followed by γ-CD and then α-CD [15]. The absorption and ^1H NMR spectra for chromone and flavone in the presence of β-CD showed that both ketones form complexes with this CD. Chromone binds less efficiently to β-CD than xanthone (Table 2). The smaller equilibrium constant observed for chromone can be explained by the smaller size of this ketone when compared to xanthone or flavone. Indeed, the magnitude of the decrease in the binding efficiency observed for chromone when compared to xanthone (ca. 4.6) is similar to the decrease when benzene is compared to naphthalene (ca. 4.9, for averaged values in the equilibrium constant compilation in [2]).

The equilibrium constants for xanthone and flavone are the same within experimental errors. Theoretical studies

Table 2
Equilibrium constants for the ground-state and excited triplet states of guest molecules with β-CD

Guest	$K_{ground\text{-}state}$ (M^{-1})	$K_{triplet}$ (M^{-1})
Xanthone	1100±200[a]	48±13[a] 90±10[b]
Chromone	240±40	140±50[b]
Flavone	1090±80	550±350[b]
1-Naphthyl-1-ethanol	500±80[c]	980±540[d]
2-Naphthyl-1-ethanol	1800±200[c]	1600±1100[d]

[a] From [16].
[b] Calculated as the ratio from k_+ and k_- obtained for the quenching with Cu^{2+}.
[c] From [19] in the presence of 0.5 M Na_2SO_4.
[d] Calculated from k_+ and k_- in [19].

combined with induced circular dichroism measurements showed that xanthone interacts with β-CD at the rim of the cavity containing the secondary alcohols [36]. One of the aromatic rings of xanthone is included into the β-CD cavity, whereas the second ring is fairly exposed to the aqueous phase. This mode of interaction minimizes water contact with the hydrophobic surface of xanthone without leading to the breakage of all hydrogen bonds to the xanthone oxygen atoms. Flavone contains two moieties that can interact with the β-CD cavity, i.e. the chromone and phenyl rings. Two modes of interaction with β-CD in complexes with 1:1 stoichiometries could occur. One possibility is the interaction of the chromone moiety with the cavity leaving the phenyl ring exposed to the aqueous phase, whereas in the second mode the phenyl ring is deeply included into the CD cavity and the chromone portion interacts with the rim. The value for the equilibrium constant of flavone with β-CD does not provide any information on the mode of interaction and we do not have any information from the absorption or NMR experiments that could be used to differentiate between these two modes of interaction.

The higher complexation efficiency of flavone compared to chromone could also be due to the formation of a 1:2 (flavone:β-CD) complex. However, the double-reciprocal plots (Benesi–Hildebrand treatment) for the absorbance changes of flavone in the presence of β-CD were consistent with a 1:1 binding stoichiometry. If the 1:2 complex contributed significantly to the fraction of complexed flavone we would have expected the double reciprocal plot to deviate from linearity at high CD concentrations. However, if the binding of the second β-CD molecule was weak only a small portion of flavone could be bound to two CDs and the formation of this complex may not be detectable in the absorption experiments performed.

The dynamics of complexation to β-CD was studied for the triplet states of flavone and chromone. The triplet–triplet absorption spectra for these ketones in water were similar to those observed in acetonitrile [26,30,31], and no formation of ketyl radicals that absorb at 500 nm [30,31] was observed. Both these ketones have low lying triplet states with n,π* and π,π* character [27,37]. However, the maxima for the triplet–triplet absorption did not significantly shift with solvent polarity as is observed for triplet xanthone [21]. For this reason, we employed the quenching methodology to determine the complexation dynamics of these ketones with β-CD. The triplet excited states of xanthone, flavone and chromone in water were quenched by Cu^{2+} ions and the recovered quenching rate constants were similar (within a factor of 2), suggesting that the same quenching mechanism operates for all three ketones.

All ketones in water were easily photoionized at the laser energies employed. The solvated electron absorption interfered with the excited triplet absorption of the ketones above 600 nm. We employed N_2O to trap the solvated electrons. The addition of N_2O was preferred over decreasing the laser energy, since the later procedure leads to a decrease of the signal-to-noise ratio. However, caution should be exercised when adding nitrous oxide since its reaction with solvated electrons leads to the formation of hydroxyl radicals [24] and N_2O has been shown to trap radicals [38]. In our experiments, N_2O did not interfere with the triplet states since the triplet lifetimes in the presence of N_2O or N_2 were the same.

Eq. (4) can be employed to fit the k_{obs} versus quencher concentration plots when the concentration of excited probe in the aqueous phase is much lower than the complexed excited state concentration. Since only small changes were observed in the ground state absorption spectra of the ketones with the addition of β-CD, we can assume that the initial excited state concentration of free and complexed ketone can be calculated from the ground state equilibrium constants. In all cases more than 90% of the ketones were complexed and the assumption that the concentration of free probe is small is valid. In addition, the triplet kinetics at all quencher concentrations followed a mono-exponential decay, which is a condition required for the application of Eq. (4).

The quenching in the presence of β-CD by Cu^{2+} of all ketones and the quenching of flavone and chromone by NO_2^- clearly led to a downward curvature of the quenching plots (Figs. 4 and 5). This result indicates that β-CD protects the triplet ketones from the ionic quenchers in the aqueous phase. The curvature of the quenching plots suggests that the exit of the triplet states from the β-CD cavity is the rate limiting process for quenching to occur. It is worth noting that the deviation from linearity is more pronounced for flavone than for chromone for both quenchers employed. This result shows that exit of triplet flavone from β-CD is slower than the exit for chromone. Unfortunately for none of the quenching plots a high enough ion concentration could be employed to achieve the linear region predicted by Eq. (4). Consequently, when k_{qH}, k_+ and k_- were left as free parameters during the fitting of the experimental data to Eq. (4) a wide range of recovered values led to adequate fits. These recovered values included negative values for k_{qH}; a result that has no physical meaning. The value for k_{qH} has to lie between 0 and k_{qo}. The downward curvature in the quenching plots indicates that k_{qH} has to be smaller than k_{qo}, because if both values were equal a linear quenching plot would have been observed. For this reason, several fits were employed by incrementally increasing k_{qH} until the fits deviated significantly from the experimental data. In the case of Cu^{2+} quenching this procedure led to acceptable values for the exit and entry rate constants of all three ketones. The precision is better for the exit rate constants than for the entry rate constants. This is a consequence of the dependence of k_{obs} with the quencher concentration (Eq. (4)), where the exit rate constant contributes to the curvature at moderate quencher concentration but also determines the linear portion of the plot at high quencher concentrations. In contrast, the entry rate constant is only determined by the curvature of the quenching plot. In the case of nitrite quenching, the experiments were limited by the time-resolution of the laser flash photolysis set-up, due to the much higher quenching

efficiency of NO_2^- when compared to the quenching by Cu^{2+}. For this reason, the nitrite quenching experiments do not lead to quantitative values for the entry and exit rate constants.

The complexation dynamics of xanthone with β-CD was studied using the quenching methodology, because the objective of this work was to compare the entry and exit rate constants for xanthone, flavone and chromone. The entry and exit rate constants for xanthone were previously determined by spectroscopically following the exit of xanthone from the CD cavity after the excitation of the ketone to its triplet state [16,18]. Undoubtedly this direct methodology leads to more accurate values for k_+ and k_-, because no mechanistic assumptions have to be made. However, for most probes the quenching methodology has to be employed and for this reason it was important to compare the recovered values for the entry and exit rate constants using both methodologies. The quenching methodology clearly overestimates the values for k_+ and k_- (Table 1). This effect is more pronounced for the entry rate constant than for k_-. The reasons for these differences are not understood at this point. This result suggests that relative comparisons for the dynamics of guest complexation with CDs recovered from quenching studies can be made, if one keeps in mind that the entry rate constants are overestimated to a greater extent than the exit rate constants.

The entry rate constants for all three ketones are similar if we consider the experimental uncertainties. These entry rate constants are smaller than the diffusional controlled limit of bimolecular reaction in water ($6.5\times10^9\,M^{-1}\,s^{-1}$) [35], suggesting that the directionality between the CD cavity and the guest in the encounter complex is important for complex formation. The k_+ values for the ketones are higher than those observed for naphthylethanols. It is worth noting that different quenchers were employed for the determination of k_+ for the ketones and the naphthylethanols. However, the difference observed for the k_+ values is not due to the nature of the quencher, because in the case of naphthylethanols similar k_+ values were recovered using Mn^{2+} or nitrite as quenchers [19]. Guest entry into CD cavities involves the desolvation of the guest and the desolvation and structural change of the CD to accommodate the guest molecule. Triplet excited ketones with π,π* configuration (xanthone and flavone) will have a higher basicity and dipole moment when compared to their ground-states. In contrast, excited naphthylethanols will have a higher dipole moment in the excited state but the basicity of the molecule will not change appreciably. A lower entry rate constant would be expected for the ketones when compared to the naphthylethanols, if desolvation of the guest was the rate limiting step for complex formation. Therefore, the higher k_+ values for the ketones have to be related to less CD desolvation or smaller structural changes when the complex with the ketones is formed. Indeed, for xanthone it was shown that this guest only interacts with the rim of the cavity [36]. In the case of chromone the large entry rate constant observed could be due to the fact that this molecule is small and interaction with the β-CD does not lead to complete desolvation of the CD cavity.

The exit rate constants of guests from the CD cavity are much more sensitive to the structure of the guests. The k_- value is highest for chromone followed by xanthone and then flavone. Size is definitely a factor because the smallest guest has the fastest exit rate constant. In addition, the significant difference observed for the xanthone and flavone exit rate constants is in contrast to the ground-state complexation where both molecules have similar equilibrium constants. The smaller exit rate constant for flavone could be related either to a lower basicity of the triplet excited state of flavone or a different type of complex with the β-CD when compared to xanthone. The ground state pK_a values for xanthone, chromone and flavone are respectively −4.1 [39], −2.0 [40] and −1.33 [41]. In the case of xanthone the excited triplet state has a significantly higher basicity (pK_a=+3.0 [39]) than the ground state. The pK_a of triplet flavone is also expected to increase because it has the same π,π* configuration as triplet xanthone. The lower k_- value for flavone could only be explained by a change in basicity if the pK_a increase for flavone is much smaller than for triplet xanthone. Although the pK_a of triplet flavone is not known one would expect the increase to be similar to that observed for xanthone. For this reason, the more likely explanation for the lower exit rate constant for flavone is that its complex with β-CD is different from that observed for xanthone. Two possibilities exist for the CD complexation of flavone that would lead to a slow down of the triplet flavone exit. The phenyl ring of flavone could be deeply included in the β-CD cavity, whereas the chromone ring of flavone would sit at the rim. In this case the phenyl ring would be acting as an anchor for the guest slowing down its exit. This mode of incorporation is not in contradiction with a fast entry rate constant, since the phenyl ring is relatively small and its incorporation into the β-CD cavity does not require much rearrangement of the cavity's structure. The second possibility is that the chromone ring of flavone would be more deeply included in the CD cavity than in the case of xanthone and the phenyl ring would interact with the rim of the CD. In this case the phenyl ring would as act as a cap to slow down the flavone exit. Although we do not have any experimental evidence to differentiate between these possibilities, the one in which the phenyl ring acts as an anchor seems more likely based on the relative hydrophobicities of the phenyl and chromone moieties in flavone.

The exit rate constants of the ketones are at least one order of magnitude larger than the k_- values observed for the naphthylethanols. Since both types of molecules have low lying triplet states with π,π* configuration the triplet excited states have higher dipole moments than the ground states. The large difference in the exit rate constants suggests that the larger driving force for exit in the case of the ketones is not the higher dipole moment of the excited state, but its higher basicity. This conclusion is also supported when the equilibrium constants for the triplet excited state, calculated

from the ratio of k_+ and k_-, are compared to the equilibrium constants for the ground states (Table 2). It is worth noting that the calculated $K_{triplet}$ are probably upper limits due to the overestimation of the k_+ values when the quenching methodology is employed (see above). In the case of the triplet ketones the equilibrium constants for the triplet states are significantly lower than the ground state equilibrium constants, whereas in the case of the naphthylethanols such a decrease was not observed.

In conclusion, the use of flavone and chromone as guest molecules for complexation with β-CD showed that the complexation dynamics depends on the structure of the guest molecules. Aromatic ketones have larger entry and exit rate constants than aromatic hydrocarbons substituted with an alcohol functionality. The faster complexation dynamics was attributed to the higher basicity of the ketones. In addition, we showed that the exit rate constant is much more sensitive to the structure of the guest than the entry rate constants.

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