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Fluorescence enhancement of curcumin upon inclusion into parent and modified cyclodextrins

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Abstract

The effect of α-, β-, and γ-cyclodextrin (CD) and their hydroxypropylated (HP) derivatives on the solubility and fluorescence of the compound curcumin has been studied. Curcumin, the main constituent of the Indian spice turmeric, is of growing interest due to its wide-ranging pharmaceutical properties. All of these six cyclodextrins significantly increase the aqueous solubility of curcumin, with the greatest solubility observed in HP-γ-CD. Curcumin forms 2:1 host–guest inclusion complexes with these cyclodextrins, with the strongest complexes formed in the case of HP-β-CD. These 2:1 complexes are postulated to form when a cyclodextrin host encapsulates each of the two phenyl rings at the ends of the curcumin molecule. The equilibrium constant for encapsulation by the second cyclodextrin host is significantly smaller than that for the first in each case, probably a result of steric bulk of the first cyclodextrin, with deep inclusion of the curcumin molecule, hindering encapsulation by the second cyclodextrin host. In fact, in the case of β-CD, a 1:1 host:guest model fit the data just as well, or better than, the 2:1 model. In the case of γ-CD and HP-γ-CD, the initial 1:1 complex formed was found to be *less* fluorescent than the free curcumin; this is postulated to be a result of inclusion of a folded curcumin molecule, which breaks the conjugation along the length of the molecule and hence greatly reduces its fluorescence quantum yield.

Keywords: Cyclodextrins; Curcumin; Fluorescence enhancement; Host–guest inclusion

1. Introduction

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) [1–7], shown in Fig. 1a, is a fascinating molecule. It is the main constituent of the Indian spice turmeric, and gives curry sauces their characteristic yellow colour. It is a fluorescent molecule, with emission properties highly dependent on the polarity of its environment [8–10]. However, the most intriguing aspect of this compound is that it shows a wide array of pharmaceutical activity, including potent antioxidant, anti-inflammatory, and anti-carcinogenic properties [1–7]. Unfortunately curcumin has an extremely low aqueous solubility, limiting its pharmaceutical use. One possible way to increase its aqueous solubility is to form inclusion complexes, i.e. to encapsulate curcumin as a guest within the internal cavity of a water-soluble host. Two recent studies of the inclusion complexes of curcumin with the common host compounds cyclodextrins (CDs) have been reported [11,12]. Tang et al. [11] used absorption spectrophotometry to study the supramolecular host–guest inclusion of curcumin into β-cyclodextrin, and reported a strong 2:1 host:guest complex with an apparent formation constant of $5.53 \times 10^5\ M^{-2}$. Tønnesen et al. [12] investigated the inclusion of curcumin into the three common cyclodextrins, α-, β- and γ-, as well as their hydroxypropylated derivatives, using relative hydrolysis rates. In addition, they measured the solubility enhancement of the modified CDs on curcumin in aqueous solution. However, they assumed 1:1 host:guest inclusion (which is not an obvious stoichiometry given the two identical phenyl groups at either end of the curcumin molecule which are available for binding), and only determined minimum and/or estimated association constants.

We recently reported fluorescence studies of the inclusion complexes of curcumin with another family of hosts, namely the macrocyclic compounds cucurbit[*n*]uril (Qn) [13]. Strong 2:1 host:guest complexation was observed, with a

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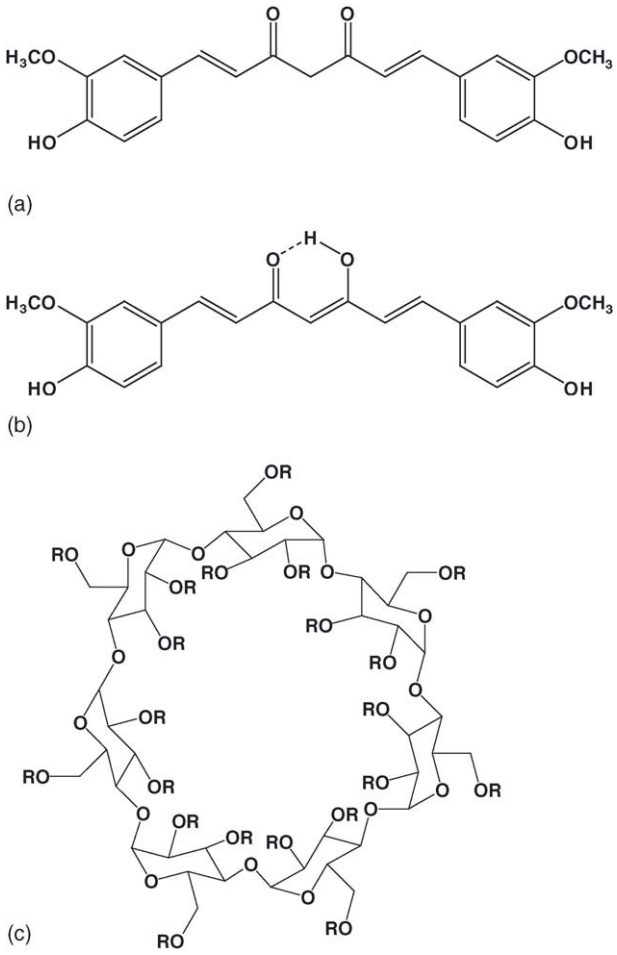

Fig. 1. Molecular structures of the guest and hosts: (a) curcumin (diketone form); (b) curcumin (enol form); (c) β-cyclodextrin (R = H) or HP-β-cyclodextrin (R = H or $CH_2CHOHCH_3$).

high enhancement of the curcumin fluorescence, however only with cucurbit[6]uril (Q6), and not with the larger cucurbit[7]uril (Q7), contrary to expectations. Furthermore, 2:1 inclusion was observed, which was postulated to be the result of inclusion of both phenyl ends of the curcumin by host molecules, similar to that reported for β-CD by Tang et al. [11] but contrary to the 1:1 inclusion assumed by Tønnesen et al. [12] for CD inclusion.

In light of the large observed dependence of binding efficiency on cavity size in the case of the cucurbituril host, and the inconsistency in the literature with respect to complex stoichiometry, we decided to further investigate the effect of cyclodextrin size on curcumin complexation. In this paper, we use the highly sensitive fluorescence spectroscopic technique to study the inclusion complexes of curcumin with parent and hydroxypropylated α-, β-, and γ-CD. This technique allows for the accurate determination of host–guest stoichiometry and association constants (which have only been reported accurately in the case of β-CD [11]), as well as for the study of the effect of cyclodextrins on the emission properties of this very interesting fluorescent probe molecule (which has not been previously investigated).

2. Experimental

2.1. Materials

The following compounds were obtained from the indicated sources and used as received: curcumin, α-CD, β-CD, HP-α-CD, HP-β-CD, and HP-γ-CD were obtained from Aldrich Chemical Co.; γ-CD was obtained from Cerestar, USA. In the case of the HP-substituted CDs, these commercial products are heterogeneous, with a range of number of HP substituents and substitution pattern. On average, the degree of substitution (in terms of the average number of HP substituents per monomer unit) was 0.6, 1.0 and 0.6, for HP-α-CD, HP-β-CD and HP-γ-CD, respectively (the highest available in each case). Tests of the water content of the cyclodextrins showed values ranging from 3.3 to 11.8% for all of the cyclodextrins used (based on mass loss after heating for 4 h in a vacuum oven at 180 °C). The cyclodextrins were not dried before use, however the calculated cyclodextrin concentrations were corrected using the determined water content values.

2.2. Solution preparation

For solubility measurements, saturated solutions were prepared by adding an excess of curcumin to the appropriate solution, sonicating for 30 min, then allowing to equilibrate in the dark overnight. Reference solutions for the solubility calculations containing exactly 2.00×10^{-5} M curcumin were prepared in 10 mM HP-β-CD and HP-γ-CD by weighing out the appropriate amount of both curcumin and the CD of interest into a 10.00 mL volumetric flask, and filling to the mark with distilled water.

For all fluorescence experiments, the appropriate amount of the CD of interest was weighed into a 5 mL glass vial, then dissolved by adding 3.00 mL of nanopure water. A volume of 30 μL of a 1.00×10^{-3} M curcumin stock solution in methanol was then added, giving a constant curcumin concentration of 1.00×10^{-5} M in 1% methanol/water for all fluorescence measurements. These solutions had an absorbance of 0.36 ± 0.02 at 425 nm at all CD concentrations used. The solution was shaken, then transferred to a fluorescence cuvette for spectroscopic measurements.

2.3. Fluorescence spectroscopy

All absorption and fluorescence measurements were performed on solutions in 1 cm^2 quartz cuvettes at 22 ± 1 °C. Absorption spectra were measured on a Cary 50 Bio UV–vis Spectrophotometer. Fluorescence spectra were measured on a Photon Technology International LS-100 luminescence spectrometer, with excitation and emission monochrometer

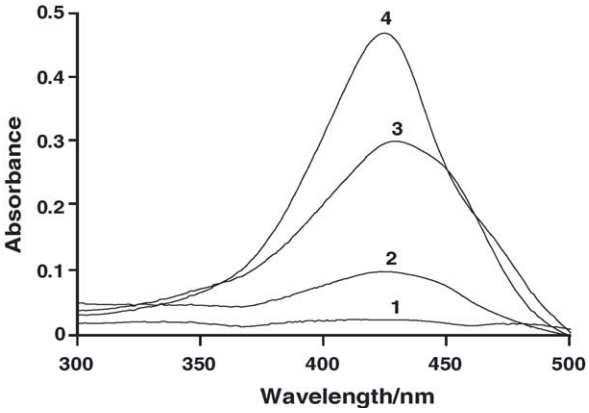

Fig. 2. Absorption spectra of saturated solutions of curcumin in various aqueous solutions: (1) no CD; (2) 10 mM β-CD; (3) 10 mM HP-β-CD; (4) 10 mM HP-γ-CD.

bandpasses set at 3 nm and an excitation wavelength of 425 nm. The fluorescence enhancement F/F_0 was determined at each CD concentration as the ratio of the integrated fluorescence spectrum in the presence of the CD to that in its absence. For each CD, at least three independent trials were performed, with the results averaged at each [CD], and the averaged results fit to the appropriate equations to extract association constants (as described in Section 3.3) using nonlinear least squares fitting procedures.

3. Results and discussion

3.1. Aqueous solubility enhancement

Tønnesen et al. [12] have reported the effect of modified cyclodextrins on curcumin solubility, but did not report that of the parent cyclodextrins. Thus, the effect of all six cyclodextrins (parent and hydroxypropylated) on the aqueous solubility of curcumin was studied by preparing saturated solutions of curcumin in 10 mM (the maximum β-CD solubility) cyclodextrin solutions. The increased solubility in the presence of the cyclodextrins was monitored by measuring the curcumin absorption spectrum of these solutions. The spectra are shown in Fig. 2, which shows two important results. First, the absorbance of the saturated solution increases significantly in the presence of cyclodextrins, indicating an increase in solubility (assuming no significant effect of the cyclodextrins on the extinction coefficient – see later). Second, there is only a slight spectral shift observed, which is consistent with previous results indicating a very small solvent polarity effect on the absorption (contrary to fluorescence) spectrum of curcumin [8]. Table 1 lists the absorbance of these solutions at the maximum absorption wavelength of 425 nm. These results indicate that the modified cyclodextrins are better at solubilizing curcumin than are the corresponding parents (consistent with previous reports on cyclodextrin enhancement of solubility [14,15]), and that in the case of

Table 1
Absorbance at 425 nm of saturated curcumin in 10 mM cyclodextrin solutions

Solution	A_{425}
No CD	<0.001
α-CD	0.092
HP-α-CD	0.12
β-CD	0.099
HP-β-CD	0.30
γ-CD	0.070
HP-γ-CD	0.47

the parent cyclodextrins, β-CD gives the largest solubility, whereas in the case of the hydroxypropyl cyclodextrins, HP-γ-CD gives the largest solubility. This would suggest that β-CD has the best match in cavity size to curcumin, and that the γ-CD cavity is too large. However, the hydroxypropyl side chains in HP-γ-CD must become involved in the inclusion process, holding the curcumin in the large cavity.

In the case of HP-β-CD and HP-γ-CD (which showed the largest enhancement of solubility), these results were quantified by preparing 2.00×10^{-5} M reference curcumin solutions in 10 mM solutions of each these two cyclodextrins, and then using Beer's Law and the absorbances of the saturated and reference solutions to determine the concentration of the saturated solutions. This procedure gave extinction coefficients of 5800 and 3100 $M^{-1}\,cm^{-1}$, and solubilities of 5.2×10^{-5} and 1.4×10^{-4} M, for 10 mM HP-β-CD and HP-γ-CD solutions, respectively. The aqueous solubility of curcumin has been reported to be $<3 \times 10^{-8}$ M [12]; these values thus represent increased solubilities of factors of at least 1700 and 4700 for HP-β-CD and HP-γ-CD, respectively. These solubilities are consistent with those reported by Tønnesen for hydroxypropylated cyclodextrins at higher concentration: 1.15×10^{-4}, 1.22×10^{-4} and 3.82×10^{-4} M for 83 mM HP-α-CD, 71 mM HP-β-CD and 63 mM HP-γ-CD, respectively [12]. It is interesting to note however the significant difference in extinction coefficient of curcumin in the HP-β-CD and HP-γ-CD solutions: that in HP-β-CD is nearly twice as large as that in HP-γ-CD. This means that the increased solubility in HP-γ-CD relative to HP-β-CD is even greater than that indicated by the increase in absorption spectrum illustrated in Fig. 2. This difference in extinction coefficient in the two cyclodextrins will be further discussed later.

3.2. Fluorescence enhancement

All of the cyclodextrins studied had significant effects on the fluorescence spectrum of curcumin, both in terms of peak position and intensity. For example, the effect of various concentrations of HP-β-CD on the curcumin fluorescence spectrum is shown in Fig. 3. In this figure, a significant blue shift and very large enhancement of the curcumin fluorescence with increasing HP-β-CD concentration can clearly be observed. Table 2 lists the observed wavelength of maximum

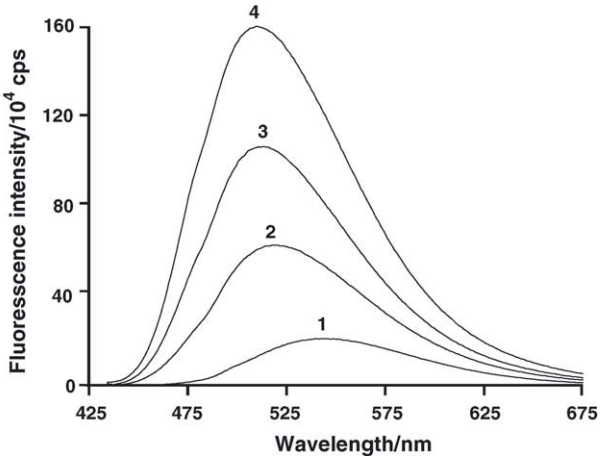

Fig. 3. Fluorescence spectra of 1.00×10^{-5} M curcumin in 1% methanol/water in the presence of various amounts of HP-β-CD: (1) 0 mM; (2) 1 mM; (3) 10 mM; (4) 30 mM.

emission of the spectrum, $\lambda_{F,max}$, as well as the enhancement, F/F_0, for all of the cyclodextrins studied, at 10 mM concentration. For both the parent and modified CDs, β-CD gave a larger enhancement than did α-CD, suggesting a better size match of curcumin with β-CD. The large cavity in the case of γ-CD gave some very surprising results, however: in the case of parent γ-CD, a larger enhancement was observed than in the case of β-CD, whereas in the case of the modified CDs, HP-γ-CD actually showed a *decrease* in fluorescence. Similar trends can be seen in comparing $\lambda_{F,max}$: a continual blue-shift is observed as the cavity size is increased and upon moving from parent to modified CD, indicating that curcumin is experiencing an increasingly less polar cavity upon inclusion into this series of CDs. Again, the exception to this trend is HP-γ-CD, which showed the *smallest* blue shift relative to free curcumin. These trends, and the anomalous results for HP-γ-CD, will be further addressed in the next section.

As can be seen from Table 2, HP-β-CD gives the strongest enhancement, of a factor of 5.7 at 10 mM and a maximum measured enhancement of a factor of 7.1 at 30 mM. This is higher than the enhancement of 5.1 reported for 40 mM Q6, but still is lower than the polarity sensitivity factor, PSF, of 39 measured for curcumin [13]. The PSF is the relative fluorescence of a probe in ethanol as compared to aqueous solution; thus curcumin included in the HP-β-CD cavity is experiencing a polarity significantly higher than that of ethanol. This is in contrast to the case of the fluorescent probe 1,8-ANS, which showed maximum enhancement upon inclusion into HP-β-CD very similar to the measured PSF [16]. This suggests that curcumin is not as completely included in the HP-β-CD host than was the case with 1,8-ANS.

The fact that these experiments are done in 1% by volume methanol/water solution does allow for the possibility that methanol is being co-included with curcumin in the cyclodextrin, giving a ternary complex. To test for this, the fluorescence of curcumin in 2 mM and 10 mM HP-β-CD solutions was measured at three methanol concentrations: 1, 2 and 3%. There was no significant change in the curcumin fluorescence intensity as a function of methanol concentration (the integrated intensity varied by less than 1% for both cyclodextrin concentrations), thus it can be concluded methanol does not play a role in the complexation process.

Table 2
Effects of various cyclodextrins (10 mM) on the maximum emission wavelength ($\lambda_{F,max}$) and integrated intensity (F/F_0, relative to no CD) of the fluorescence spectrum of 1.00×10^{-5} M curcumin in 1% methanol/water

CD	$\lambda_{F,max}$ (nm)	F/F_o
None	543	1.0
α-CD	524	2.1
HP-α-CD	520	3.0
β-CD	519	3.1
HP-β-CD	511	5.7
γ-CD	509	3.5
HP-γ-CD	532	0.25

3.3. Association constants

The fluorescence enhancement F/F_0 measured as a function of host concentration can be used to obtain the association constant(s) for the host–guest inclusion process. In the case of 1:1 host:guest inclusion, a single equilibrium is involved, with association constant K:

$$\text{CD} + \text{curcumin} \rightleftharpoons \text{CD:curcumin} \quad (1)$$

$$K = [\text{CD:curcumin}]/([\text{CD}][\text{curcumin}]) \quad (2)$$

In this case, the dependence of F/F_0 on added host concentration, $[CD]_0$, is given by the following equation [17,18]:

$$\frac{F}{F_0} = 1 + \left(\frac{F_\infty}{F_0} - 1\right)\frac{[\text{CD}]_0 K}{1 + [\text{CD}]_0 K} \quad (3)$$

where F_∞/F_0 is the maximum enhancement, when all guests are complexed within a host. If only 1:1 complexes are formed, then the double-reciprocal plot of $1/(F/F_0 - 1)$ versus $1/[CD]_0$ will be linear; a non-linear double-reciprocal plot indicates the presence of higher-order inclusion complexes.

Considering the elongated, symmetrical shape of curcumin, 2:1 host:guest complexation in which each curcumin guest is complexed by a CD host at each end of the molecule, as illustrated in Fig. 4, would seem the most likely inclusion

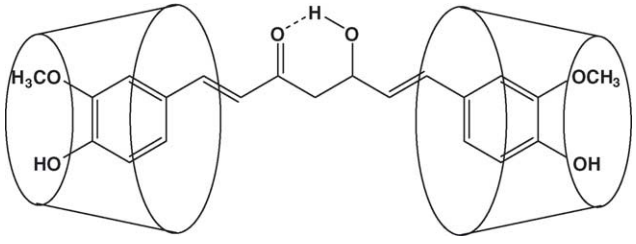

Fig. 4. The proposed structure of the 2:1 CD:curcumin inclusion complex. Note that this is a cartoon depiction only, and that the relative size of curcumin and β-CD is indicated.

Table 3
Results of the fit of the F/F_0 data for 1.00×10^{-5} M curcumin in 1% methanol/water in the six cyclodextrins to Eq. (7) for 2:1 complexation

CD	F_a	K_1 (M^{-1})	F_b	K_2 (M^{-1})	K_1K_2 (M^{-2})
α-CD	1.7 ± 0.1	3300 ± 1200	10 ± 2	5.4 ± 1.3	18000
HP-α-CD	2.0 ± 0.1	12000 ± 3000	7.3 ± 1.1	28 ± 2	340000
β-CD[a]	3.6 ± 1.2	280 ± 120	8.9 ± 7.8	6.6 ± 5.2	1800
HP-β-CD	3.0 ± 0.8	3400 ± 1800	8.0 ± 0.7	120 ± 40	410000
γ-CD[b]	(0.0)	(8000)	(6.0)	(140)	(1100000)
HP-γ-CD	0.21 ± 0.01	21000 ± 1200	0.72 ± 0.10	8.4 ± 2.2	180000

[a] β-CD data fit to 1:1 model: $F/F_0 = 4.5 \pm 0.3$; $K = 170 \pm 7\ M^{-1}$.
[b] γ-CD data fit to 2:1 model not completely satisfactory: see text and Fig. 7b.

scenario. This complexation can be described by the following stepwise mechanism, involving the initial formation of the 1:1 host:guest complex as described above in Eq. (1), followed by addition of a second host to give the 2:1 host:guest complex CD_2:curcumin:

$$\text{CD} + \text{CD:curcumin} \rightleftharpoons \text{CD}_2\text{:curcumin} \quad (4)$$

These two equilibria are described by the equilibrium constants K_1 and K_2:

$$K_1 = [\text{CD:curcumin}]/([\text{CD}][\text{curcumin}]) \quad (5)$$

$$K_2 = [\text{CD}_2\text{:curcumin}]/([\text{CD}][\text{CD:curcumin}]) \quad (6)$$

with the overall equilibrium constant K for 2:1 complexation equal to the product K_1K_2. The dependence of F/F_0 on $[\text{CD}]_0$ for this complexation mechanism is given by [19]:

$$\frac{F}{F_0} = \frac{1 + F_1/F_0 K_1[\text{CD}]_0 + F_2/F_0 K_1K_2[\text{CD}]_0^2}{(1 + K_1[\text{CD}]_0 + K_1K_2[\text{CD}]_0^2)} \quad (7)$$

where F_1/F_0 and F_2/F_0 are the fluorescence enhancement of the 1:1 and 2:1 complexes, respectively, relative to unbound guest.

The fluorescence titration results (F/F_0 versus [CD]) for the six CDs were fit to Eq. (7) using a non-linear least squares fitting program written in our lab; the results are summarized in Table 3, and illustrated in Figs. 5–7 for modified and parent α-CD, β-CD, and γ-CD, respectively. In all cases except for β-CD, non-linear double reciprocal plots were observed, indicating complexation beyond 1:1. In all cases except β-CD and γ-CD, excellent fits to Eq. (7) were obtained, in agreement with the proposed 2:1 complex formation. It should be noted that the low solubility of the guest curcumin prevents the use of the continuous variation method (or Job plot [20]), which would unambiguously determine the stoichiometry of the complex [21].

These association constant results (K_1, K_2, and K_1K_2) listed in Table 3 show some very interesting and unexpected trends. In all cases, $K_2 \ll K_1$. Thus, inclusion of one end of the curcumin molecule in a CD cavity makes inclusion of the other end of the molecule by a second CD host less likely. This is contrary to what we observed in the case of cucurbit[6]uril (Q6) [13], for which $K_2 > K_1$, indicating that inclusion of one end *increased* the affinity of the other end for inclusion. In that case, this increased affinity for the second Q6 was postulated to be a result of a change in the electronic properties of the guest induced by encapsulation by the first Q6, resulting in an increased host–guest interaction for the second host.

This reverse trend in the case of CDs could be the result of a significant difference in environments within these two cavities (Q6 versus CD). The polarity of CD cavities has been reported in terms of similarity to organic solvents; such reports have ranged from similar to ethyl acetate [22] to similar to ethanol [23–25]. Unfortunately, there has been no report of a quantitative determination of the polarity of

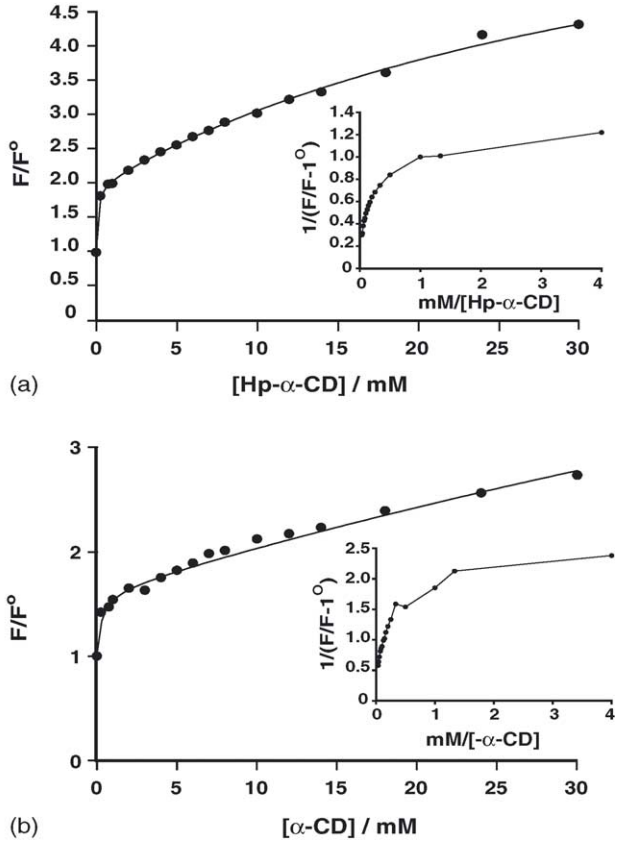

Fig. 5. The fluorescence enhancement, F/F_0, of 1.00×10^{-5} M curcumin in 1% methanol/water as a function of (a) HP-α-CD and (b) α-CD concentration. The insets show the non-linear double reciprocal plots. The solid lines show the fits of the data to Eq. (7) (2:1 complex model), using the fit parameters listed in Table 1.

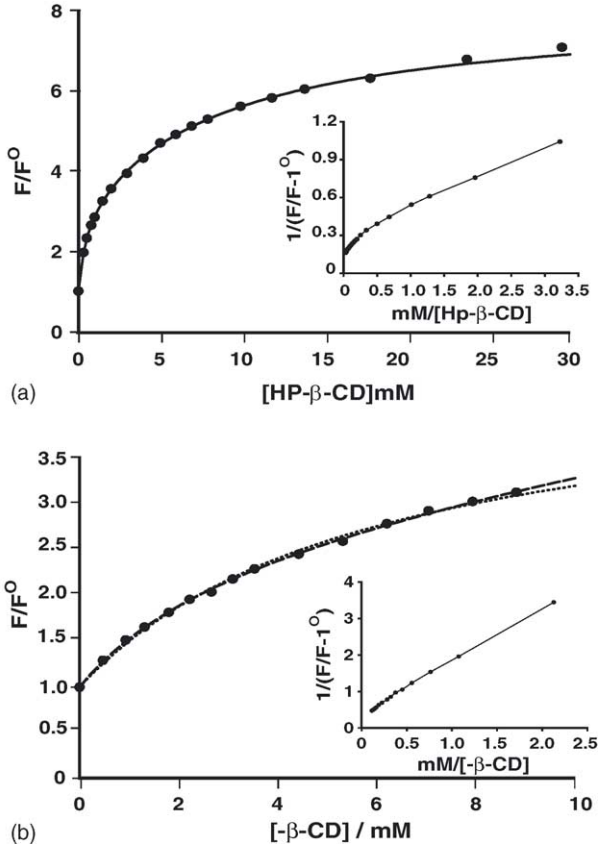

Fig. 6. The fluorescence enhancement, F/F_0, of 1.00×10^{-5} M curcumin in 1% methanol/water as a function of (a) HP-β-CD and (b) β-CD concentration. The insets show the non-linear double reciprocal plots. The solid line in (a) shows the fit of the data to Eq. (7) (2:1 complex model). The dotted line in (b) shows the fit of the data to Eq. (3) (1:1 complex model), while the dashed line shows the to Eq. (7) (2:1 complex model). The fit parameters are listed in Table 1.

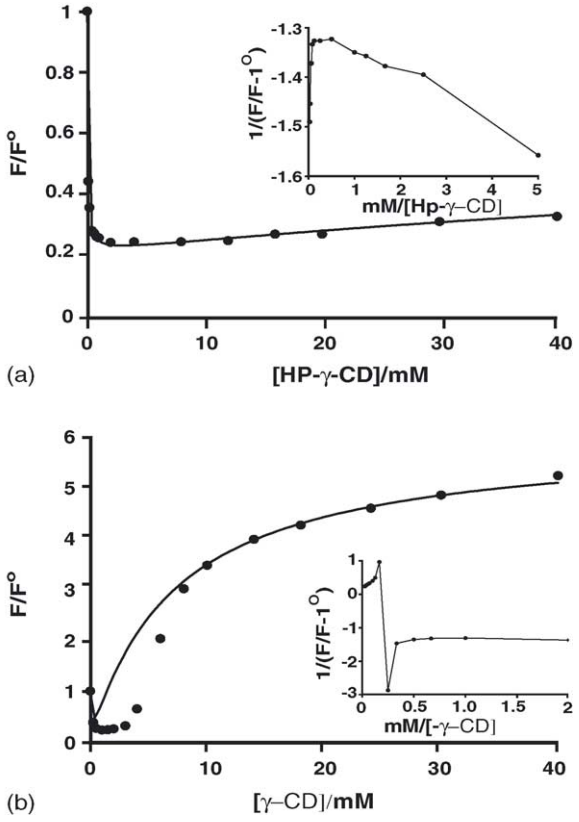

Fig. 7. The fluorescence enhancement, F/F_0, of 1.00×10^{-5} M curcumin in 1% methanol/water as a function of (a) HP-γ-CD and (b) γ-CD concentration. The insets show the non-linear double reciprocal plots. The solid lines show the fits of the data to Eq. (7) (2:1 complex model), using the fit parameters listed in Table 1.

the Q6 cavity for comparison; this is undoubtedly a result of its small portal preventing complete inclusion of polarity-sensitive probes. Nau et al. have measured the polarizability within various host cavities using the probe NBO [26,27]; they reported a polarizability inside the Q7 cavity (the homologue of Q6 with one additional monomer) of ca. 0.12, which is 50% lower than that in water, whereas that in CD cavities was reported to be similar to a 2:1 methanol:water mixture (ca. 0.21). Thus, there is a significant difference in these two cavities in terms of polarizability. However, curcumin in Q6 showed a $\lambda_{F,max}$ of 520 nm [13], exactly the same as that found here in the case of the similarly sized HP-α-CD, suggesting a similar environmental effect of the two host cavities. Thus, a more likely explanation is increased steric interactions between the two hosts encapsulating the same guest, arising from the difference in shape of CDs versus Q6. CDs are cone-shaped, with one opening larger than the other, whereas Q6 has a much more restrictive portal of carbonyl groups limiting insertion from either side, with an opening of just 4.0 Å [28]. This is significantly smaller than in the case of the CDs, which have upper rim diameters of 5.7, 7.8, and 9.5 Å for α-, β-, and γ-CD, respectively [29]. It is likely that the CD host can penetrate further along the curcumin molecule in the initial 1:1 complex, and thus interfere with encapsulation by a second CD on the other end to form the 2:1 complex.

In the case of α-CD and HP-α-CD, very strong binding was observed, both in terms of K_1, and in terms of the overall binding constant K_1K_2, with the modified CD binding significantly stronger ($K_1 = 3300$ and $12{,}000\,M^{-1}$, and $K_1K_2 = 18{,}000$ and $340{,}000\,M^{-2}$, respectively, for α-CD and HP-α-CD). A similar trend is observed with β-CD and HP-β-CD, where again the modified CD binds much more strongly that the unmodified parent ($K_1 = 280$ and $3400\,M^{-1}$, and $K_1K_2 = 1800$ and $410{,}000\,M^{-2}$, respectively, for β-CD and HP-β-CD). However, in the case of β-CD, K_2 is extremely small with a large uncertainty ($6.6 \pm 5.2\,M^{-1}$). Furthermore, as can be seen in the inset of Fig. 6b, the double-reciprocal plot is nearly linear ($r = 0.9987$), suggesting that higher-order complexes are not involved in this case. This is clearly different than the cases of α-CD, HP-α-CD, and HP-β-CD (Figs. 5a, b and 6b), for which the double-reciprocal plots are clearly non-linear, and small but significant values of K_2 are obtained. This is consistent with the proposal stated above that the larger CDs involve greater penetration and

therefore greater steric interaction between the hosts at each end of the curcumin molecule: this would clearly be a greater effect for β-CD than α-CD. It is interesting to note however that this trend with the larger CD is reversed for HP-β-CD, which actually shows the *largest* value of K_2, indicating that the increased binding affinity resulting from the addition of the hydroxypropyl chains overcomes the increased steric hindrance of attaching two hosts to a single curcumin.

An important comparison can be made between the results for α- and β-CD: binding is much stronger with α-CD than with β-CD. This indicates that there is a much better size match between the phenyl groups of curcumin and the smaller α-CD cavity. This result is consistent with what we previously reported in the case of cucurbit[*n*]urils, namely that whereas Q6 showed strong binding to curcumin, the larger Q7 did not bind at all [13]. This trend of α-CD binding more strongly than β-CD is not maintained in the case of the HP-modified CDs, however: although K_1 is significantly larger for HP-α-CD than for HP-β-CD, K_2 is much larger for HP-β-CD, giving very similar overall binding constants (K_1K_2).

A very different and totally unexpected result was obtained in the case of γ-CD and HP-γ-CD, as can be seen in Fig. 7 as compared to Figs. 5 and 6. Unlike in the cases of α- and β-CD, fluorescence suppression is observed, i.e. the presence of these two CDs actually *decreases* the curcumin fluorescence. This occurs at all concentrations in the case of HP-γ-CD, but only at low concentrations of γ-CD. However, the 2:1 inclusion model still works for these two systems, extremely well in the case of HP-γ-CD. We propose the following speculative scenario to explain these interesting and unique fluorescence titration results. As in the case of α- and β-CD, initial formation of a 1:1 complex is followed by addition of a second CD as concentration is increased, yielding a 2:1 CD:curcumin complex. The difference is that in the case of γ-CD, the much larger cavity allows for the accommodation of both phenyl ends of the guest, via a folding of the curcumin backbone. This folding would require the curcumin to be in the diketone form, with its resulting breaking of the delocalization along the entire molecule. In this form, curcumin would act as two independent half-curcumins, which have been shown to be significantly less fluorescent than curcumin [8]. This results in the initial *decrease* in curcumin fluorescence upon addition of γ- and HP-γ-CD. However, as the concentration increases, a second γ-CD includes one of the two ends of the curcumin to give a 2:1 complex with a CD encapsulating both of the phenyl ends of the molecule, analogous to the case of β-CD (as shown in Fig. 4). The curcumin can now revert to its highly fluorescent enol form, resulting in recovery and enhancement of the fluorescence in the case of the parent γ-CD. In the case of HP-γ-CD, only partial recovery is observed, indicating that the 1:1 complex is much stronger than in the case of γ-CD, making it much more difficult for a second HP-γ-CD to extract one of the two phenyl ends included in the first HP-γ-CD. In the case of HP-γ-CD, this proposed model is consistent with the good fit of the data to Eq. (7) for 1:1 followed by 2:1 complex formation, as shown in Fig. 7a. In the case of γ-CD, a less satisfactory fit was obtained with Eq. (7) (as can be seen in Fig. 7b), but the general shape of the F/F_0 versus [γ-CD] plot was reproduced by this model: an initial decrease in fluorescence ($F/F_0 < 1$) followed by a change in slope, and eventual enhancement of fluorescence ($F/F_0 > 1$).

This proposed difference in the geometry of the initial 1:1 complexes in HP-γ-CD versus HP-β-CD is supported by the absorption results described earlier: the significantly lower extinction co-efficient of the saturated curcumin solutions in the presence of HP-γ-CD as compared to HP-β-CD, and the slight but significant change in shape of the absorption spectrum (as can be seen in Fig. 2). This is consistent with our proposal that the initial inclusion in HP-γ-CD involves a folded curcumin molecule, in which the conjugation along the backbone is broken. This would be expected to result in a lowering of the extinction coefficient and a change in shape of the absorption spectrum [8]. This proposal is also supported by the results for $\lambda_{F,max}$ described earlier: curcumin included in 10 mM HP-γ-CD had the smallest spectral blue-shift relative to free curcumin (consistent with the folded geometry in the 1:1 complex), while that include in 10 mM γ-CD had the largest (consistent with a 2:1 complex with deep penetration of each end into a γ-CD cavity).

These results provide a striking illustration of the huge effect host cavity size can have on the nature of host–guest inclusion complexes. This is consistent with other reports, for example that of Dodziuk et al. [30], who observed completely different stoichiometries and degree of penetration of an adamantyl-terminated dendrimer as guest in β-CD as compared to γ-CD.

4. Conclusions

Curcumin forms strong 2:1 host:guest inclusion complexes with cyclodextrins. In the case of parent cyclodextrins, the strongest binding is observed with α-CD, the smallest cavity, whereas in the case of hydroxypropylated CDs, the strongest binding is observed with HP-β-CD. Significant enhancement of the curcumin fluorescence occurs as a result of this binding by α- and β-CDs, up to a measured maximum of a factor of 7 in the presence of 30 mM HP-β-CD. This significant enhancement could have potential applications in fluorescence-based detection methods for this important pharmaceutical compound. However, in the case of γ-CD, decreased fluorescence is observed, which has been proposed to be a result of initial inclusion of a folded curcumin molecule into the very large γ-CD cavity. In all cases, binding to these cyclodextrins provides a significant increase in the aqueous solubility of curcumin, with the largest increase of a factor of ca. 5000 in the case of 10 mM HP-γ-CD. Such large increases in curcumin aqueous solubility have potential applications in its use as a nutraceutical or pharmaceutical compound.

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