Host–guest interactions in cyclodextrin inclusion complexes with solvatochromic dyes[†]

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ABSTRACT: Host–guest interactions of α-, β-, and γ-cyclodextrin with three solvatochromic dyes derived from barbituric and Meldrum's acids, 5-(4-N,N-dimethylaminobenzylidene)-1,3-dimethyl-2,4,6(1H,3H,5H)-pyrimidinetrione (**1**), 5-[bis(4-N,N-dimethylaminophenyl)methylene]-1,3-dimethyl-2,4,6(1H,3H,5H)-pyrimidinetrione (**2**), and 5-(4-N,N-dimethylaminobenzylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (**3**), were studied by UV–visible and ^{1}H-NMR spectroscopic methods, and the obtained results compared with molecular dynamics simulations employing the linear interaction energy (LIE) protocol. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: cyclodextrins; barbituric and meldrum acid derivatives; molecular dynamics simulation

INTRODUCTION

Molecular dynamics simulations have been increasingly employed for the study of host–guest interactions in aqueous solution. Among various methods used for the estimation of affinities of ligands for proteins,[1] the linear interaction energy (LIE) protocol is a relatively straightforward option, where the solvating water molecules are treated explicitly, and not as a continuum.[2,3]

The method relies on the determination of two empirical parameters, α and β, which account for the non-polar and electrostatic contributions to the free energy of association of the enzyme complex. These parameters must be obtained from a regression analysis of a set of experimentally determined binding energies for which the LIE method has been applied.[2–4]

The method has been applied almost exclusively to the estimation of affinities of ligands to proteins.[1,5–8] The lack of applications to smaller host molecules prompted us to employ the method in a study of β-cyclodextrin (CD) inclusion complexes with two solvatochromic dyes derived from N,N-dimethylbarbituric acid.[9] Although association constants for the formation of a wide variety of CD inclusion complexes are available in the literature, the absence of corresponding simulation studies employing the LIE method precluded its use for the estimation of CD-dye binding energies. In spite of this, we showed that the method could be used for comparing different modes of inclusion of the dyes into the hydrophobic CD cavity. We could also arrive at a

qualitative comparison of the relative stabilities of these different conformers in solution. Our theoretical predictions were based on UV–visible spectral changes that did not provide enough evidence for a quantitative comparison of the complexes and their association constants. In the present work, we determined the association constants of these and related CD complexes. In addition, by means of ^{1}H-NMR spectral analysis, we were able to deduce host–guest interactions for these complexes. The experimental results were compared with the complex structure obtained by simulations employing the LIE method in a complete study of a γ-CD inclusion complex with the dye **3**.

RESULTS

UV–visible spectroscopic measurements

Formation of inclusion complexes was evident from the spectra of dye **1** in the presence of increasing concentrations of α- and γ-CDs. In the first case, a hypochromic shift of the charge-transfer maximum at 488 nm was observed (Figure 1a). In the latter case, this hypochromic shift was accompanied by the appearance of a shoulder near 450 nm (Figure 1b). In both cases, isosbestic points suggested equilibria between two species in solution.

Spectra of dye **2** exhibited small changes in the presence of α- and γ-CDs. A similar situation had rendered difficult the determination of an association constant for the complex formed between **2** and β-CD.[9] In the present work, we resorted to ^{1}H-NMR measurements for the determination of this constant (see below).

Negligible spectral changes were observed for solutions of dye **3** in the presence of α- or β-CDs.

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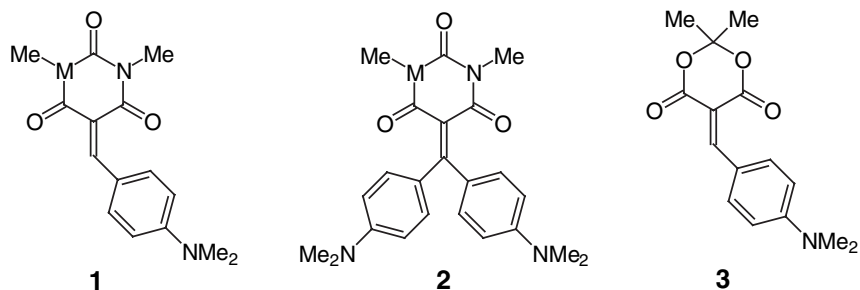
Scheme 1

Judging from these spectra, there was little association between these species. With γ-CD, more significant changes were observed but their interpretation was not straightforward. After an initial decrease of the CT absorbance at 475 nm, u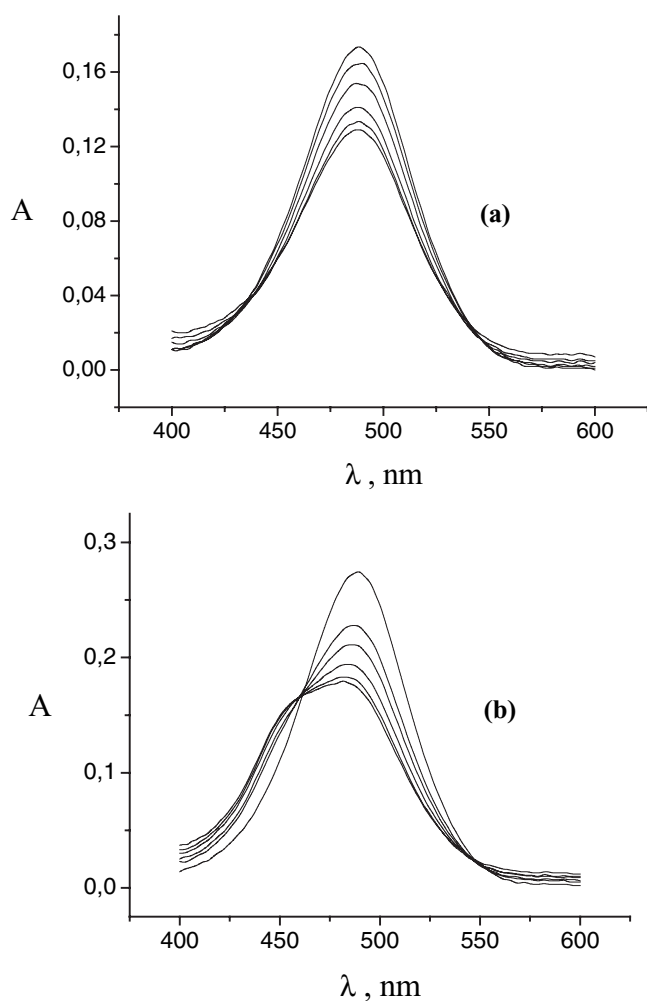pon addition of a nearly 300-fold excess of γ-CD, absorbance readings increased consistently with the addition of more CD (Figure 2). Treatment of five data points obtained for various concentrations of γ-CD, according to Eqn (3) below, yielded a straight line with a very good correlation coefficient (0.998) (Figure 3), allowing the determination of a 1:1 association constant for the γ-CD/dye **3** complex, given in Table 1. These observations led us to discard the initial absorbance decrease as an artifact, arising from difficulties of keeping guest concentrations constant in a supersaturated solution. Such problems, met with supersaturated solutions of CD inclusion complexes, are well documented in the literature.[10]

All association constants were obtained by addition of a large excess of a CD to a fixed concentration $[D]_0$ of the corresponding dye. Under these circumstances, and assuming a 1:1 association, approximation (1) holds,

$$K = [C]/[CD] \cdot ([D]_0 - [C]) \qquad (1)$$

where $[C]$ is the concentration of the complex in equilibrium with dye D, and $[CD]$ is the concentration of added CD.

The complex concentration $[C]$ may be expressed as a

Figure 1. Spectra of dye **1** in water:methanol (95:5) (dye concentration ca. 10^{-5} M) in the presence of increasing concentrations of α- (a) and γ-CD (b). Curves in (a) correspond, from top to bottom, to the following concentrations: $[\alpha$-CD$] = 0$, 2.0, 5.5, 12.0, 13.1, and 15.5 mM. Curves in (b) correspond, from top to bottom, to the following concentrations: $[\gamma$-CD$] = 0$, 0.8, 1.3, 2.4, 3.6, and 4.6 mM
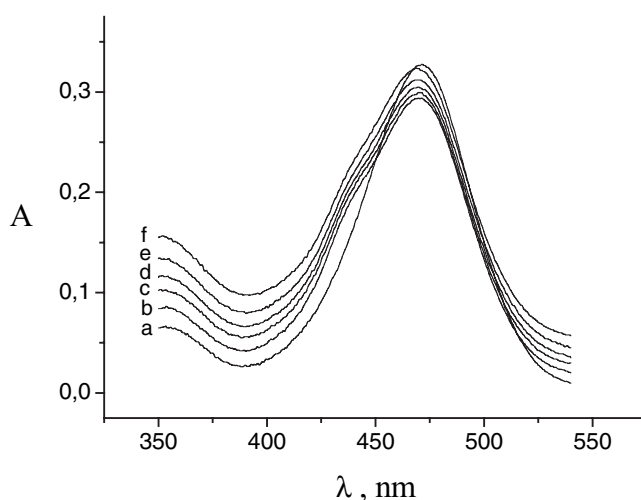

Figure 2. Spectra of dye **3** in water:methanol (95:5) ($c = 1.7 \times 10^{-4}$ M) in the presence of increasing concentrations of γ-CD, $[\gamma$-CD$] = 0$ (a), 5.1 (b), 7.7 (c), 9.3 (d), 12.8 (e), and 15.5 (f) mM
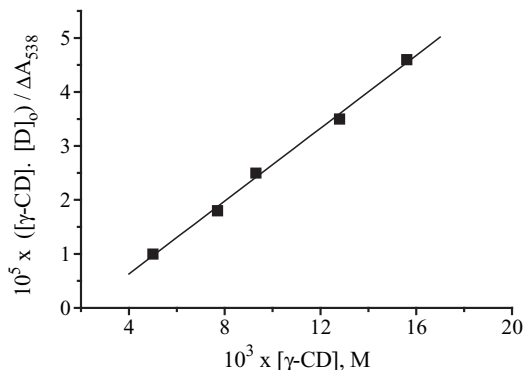
Figure 3. Benesi–Hildebrand plot for the association of dye **3** with γ-CD, according to Eqn (3)

function of absorbance variations, according to Eqn (2),

$$\Delta A = \Delta\varepsilon \cdot K \cdot [D]_0 \cdot [CD]/(1 + K \cdot [CD]) \qquad (2)$$

where $\Delta A = A - A_0$ and $\Delta\varepsilon = \varepsilon_C - \varepsilon_D$. A_0 is the absorbance of pure $[D]_0$, and the absorptivity subscripts refer to the complex C and dye D.

Equation (2) may be written as (3), allowing the association constant K to be determined by linear Benesi–Hildebrand plots of $[D]_0.[CD]/\Delta A$ *versus* [CD].

$$[D]_0 \cdot [CD]/\Delta A = 1/\Delta\varepsilon \cdot K + [CD]/\Delta\varepsilon \qquad (3)$$

An example of such a plot is shown in Figure 3, for the determination of the association constant of dye **3** with γ-CD.

Table 1 lists all the obtained association constants for dyes **1**–**3** and the investigated CDs.

¹H-NMR measurements

Because of the small variations of the UV–visible spectra of dye **2** in the presence of increasing concentrations of β-CD, we resorted to ¹H-NMR titration measurements to estimate its association constant and to investigate the form of dye insertion into the β-CD cavity. Both aminophenyl and barbituric-ring N—Me singlets exhibited shifts with the addition of the host molecule The

Table 1. 1:1 Association constants between dyes **1**–**3** and CDs

Compound	Association constant K^a, M^{-1}		
	α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
1	86 ± 1	1001 ± 84[b]	694 ± 18
2	—	153 ± 21[c]	—
3	—	—	447 ± 77

[a] Determined by UV-visible spectroscopic measurements, Eqn (3).
[b] From Ref. 9.
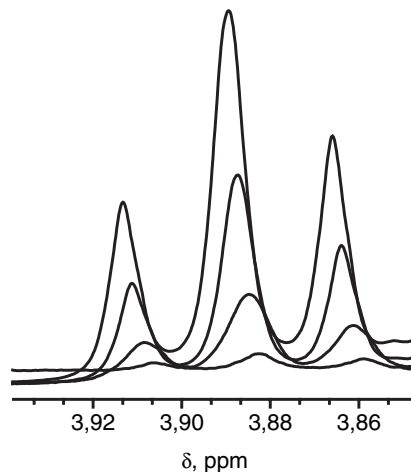[c] Determined by ¹H-NMR measurements.

Figure 4. Chemical-shift variation of the H-3 signal of β-CD for increasing concentrations of cyclodextrin, added to a 1.3×10^{-4} M solution of dye **2** in D$_2$O/CD$_3$OD 1:1. Curves correspond, in order of increasing intensities, to the following CD concentrations: 1.54×10^{-4}, 5.14×10^{-4}, 51.4×10^{-4}, and 154×10^{-4} M

small association between host and guest required large excesses of added CD, with observed shifts which were not large. Nevertheless, they were significant, when compared with other signals of the molecule, which were not affected at all. No broadening of the signals was observed, in an indication that equilibria were fast enough for the spectrometer time scale. In addition, the incorporation of the dye into the CD cavity was confirmed by chemical-shift variations of the CD H-3 and H-5 protons. Such variations are illustrated in Figure 4. Table 2 summarizes the effect of increasing concentrations of β-CD on the spectra of **2**, with chemical-shift variations of various host and guest protons.

A non-linear least-square fitting of the obtained curves of $(\delta - \delta_0)$ *versus* [β-CD] , according to Eqn (4),[11]

$$\delta - \delta_0 = \Delta\delta \cdot K \cdot [D]_0 \cdot [\beta - CD]/(1 + K \cdot [\beta - CD]) \qquad (4)$$

derived similarly to Eqn (2) above, and where $\Delta\delta = \delta_C - \delta_0$ and the subscripts C and 0 refer to the totally complexed and the free dye, respectively, allowed the determination of the 1:1 association constant K for this complex, based on the variations observed for the aminophenyl- and the barbituric-ring N—Me singlets (Figure 5). The average of these values is included in Table 1.

In contrast with the behavior of dye **2**/β-CD complex, NMR measurements of solutions of compound **3** by itself and in the presence of a 30-fold excess of γ-CD showed that the donor and acceptor fragments of this dye were affected differently by the host molecule. Significant variations were observed for the signals corresponding to the aromatic protons *ortho* (8.8 Hz) and *meta* (5.2 Hz) to the NMe$_2$ group. However, the largest observed shift (20.4 Hz) was shown by the olefinic

Table 2. Chemical-shift variations (in Hz) of some host and guest protons in the ^{1}H-NMR spectra of dye **2** in D_2O/CD_3OD (1:1 v/v), in the presence of increasing concentrations of β-cyclodextrin

$10^4 \times [\beta\text{-CD}]$, M	Cyclodextrin protons			Dye **2** protons		
	H-3	H-5	N–CH$_3$[a]	N(CH$_3$)$_2$[b]	Aromatic H[c]	Aromatic H[d]
1.5	1553.5	1519.1	1269.4	1253.2	2953.0	2722.3
84	1555.1	1522.3	1277.1	1254.9	2955.4	2716.9
154	1555.9	1523.1	1280.3	1255.4	2957.4	2711.3

[a] From the barbituric ring system.
[b] From the dimethylaminophenyl group.
[c] *meta* to the NMe$_2$ substituent.
[d] *ortho* to the NMe$_2$ substituent.

proton of the substituted benzylidene moiety. The singlet that corresponded to the NMe$_2$ group exhibited a small shift of 1.6 Hz upon addition of γ-CD. The methyl singlet of the acceptor dioxanedione fragment did not shift appreciably ($\Delta\delta = 0.4$ Hz) with the addition of the host molecule.

DISCUSSION

The ^{1}H-NMR experiments with the complexes formed between CDs and dyes **2** and **3** suggest different modes of

inclusion of these compounds into their hosts. The evidences obtained for dye **3** point to the inclusion of the whole molecule into the γ-CD cavity. The lack of sensitivity of the methyl singlets of the acceptor dioxanedione moiety or of the NMe$_2$ group to the addition of γ-CD suggests a structure where both ends of the dye molecule lie outside the hydrophobic host cavity.

A different behavior was observed for the β-CD/dye **2** system. Here both the aminophenyl and the barbituric rings were affected by the addition of the host, as shown by the fact that the N—Me barbituric and the aromatic protons *ortho* to the NMe$_2$ group shifted significantly in the presence of the CD (see Table 2). The incorporation of the dye into the hydrophobic cavity of β-CD was confirmed by chemical-shift variations of the CD H-3 and H-5 signals (Fig. 4). These observations pointed to the formation of one dye–CD complex, where the two ring systems were encapsulated within the CD cavity; or to the existence of two different complexes in fast equilibrium, with the CD cavity occupied alternatively by either ring system. The first possibility was discarded on the basis of the steric hindrance which would result from the insertion of two substituted aromatic rings into the β-CD cavity. Our previous simulations with this system supported this view and suggested the second alternative as more reasonable. Application of the LIE protocol to the two possible orientations of **2** with β-CD had suggested that they should be equally stable.[9]
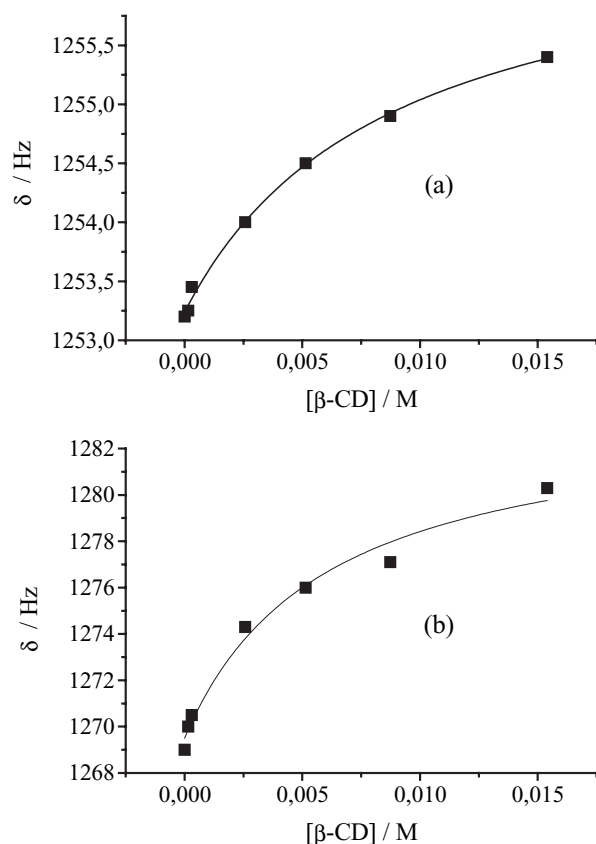
Figure 5. Variation of the chemical shifts of the aminophenyl-(a) and barbituric-ring (b) N–Me singlets of dye **2** with the addition of β-CD to a solution of **2** (1.3×10^{-4} M) in D_2O/CD$_3$OD 1:1. Fitting curves correspond to Eqn (4)
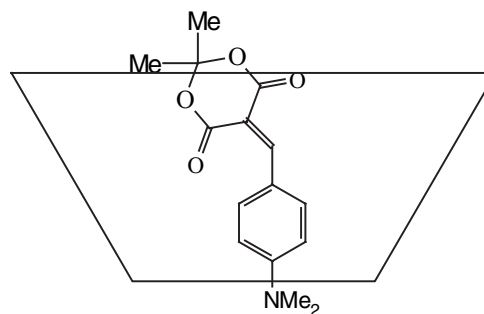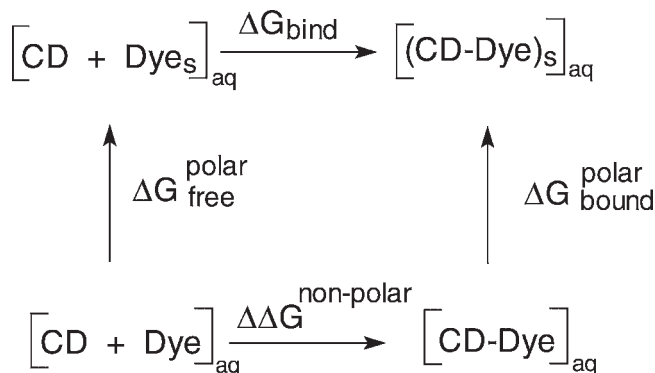

Scheme 2
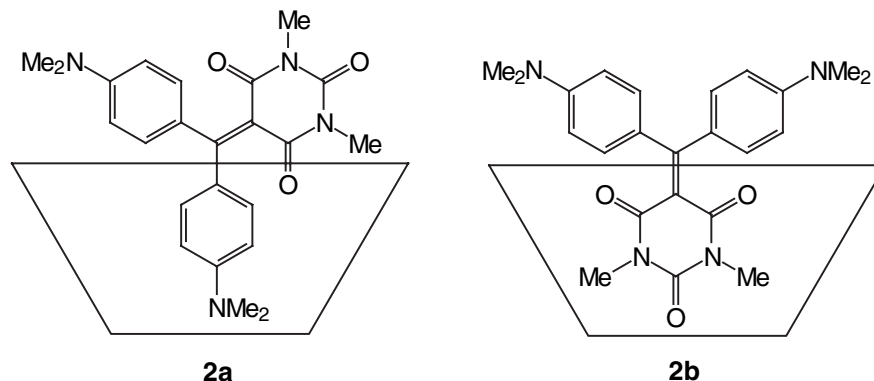
Scheme 3

Our present results support these predictions. The observation that, upon addition of increasing concentrations of β-CD, signals from the aminophenyl and the barbituric rings of dye **2** exhibit significant shifts may be reconciled with the proposal of a fast equilibrium between equally stable configurations **2a** and **2b**. As a result, both the aromatic and the barbituric rings experience the hydrophobic cavity of the CD host in the 1:1 complex.

In order to compare our conclusions based on experimental evidence, with theoretically predicted results, we carried out molecular dynamics simulations for the inclusion complex formed between γ-CD and dye **3**. We followed the same LIE protocol described previously,[9] based on the thermodynamic cycle depicted below.[2]

The binding free energy ΔG_{bind} is given by expression (5),

$$\Delta G_{\text{bind}} = \alpha(\Delta E_{\text{bound}}^{\text{non-polar}} - \Delta E_{\text{free}}^{\text{non-polar}}) + \beta \cdot (\Delta E_{\text{bound}}^{\text{polar}} - \Delta E_{\text{free}}^{\text{polar}}). \tag{5}$$

which relates this experimental value with the calculated energies ΔE. Two simulations are performed, by soaking the free dye and the bound dye–host complex in the same sphere of water molecules. Each simulation yields a non-polar and a polar term, so that the differences

Scheme 4

$(\Delta E_{\text{bound}}^{\text{non-polar}} - \Delta E_{\text{free}}^{\text{non-polar}})$ and $(\Delta E_{\text{bound}}^{\text{polar}} - \Delta E_{\text{free}}^{\text{polar}})$ measure the total non-polar and polar energy contributions, respectively, to the complexation process in water. If α and β values are available from a regression analysis of a series of experimental binding free energies and corresponding simulations, new binding energies may be estimated from theoretical simulations. We have shown that the fact that these values are not available does not preclude the comparison of different configurations for a given complex.[9] By arbitrarily assuming $\alpha = \beta = 1$ for a set of related complexes, we obtain energy values ΔE^{total} from relationship (6).

$$\Delta E^{\text{total}} = (\Delta E_{\text{bound}}^{\text{non-polar}} - \Delta E_{\text{free}}^{\text{non-polar}}) + (\Delta E_{\text{bound}}^{\text{polar}} - \Delta E_{\text{free}}^{\text{polar}}) \tag{6}$$

These values may depart significantly from the real binding energies. Nevertheless, for a given set of compounds, they may be used to compare relative stabilities and affinities within the set.

Following this procedure, we obtained the energies given in Table 3 for the γ-CD/dye **3** complex. For the sake of comparison, previously calculated energies for complexes of dyes **1** and **2** with β-CD following the same protocol[9] are also given in this Table.

The obtained structure for the γ-CD/dye **3** complex after simulation is shown in Fig. 6. The complex structure agrees very well with our expectations based on the ^{1}H-NMR analysis described above. The olefinic proton, situated in the middle of the hydrophobic CD cavity exhibits the largest chemical-shift variation. This effect is transmitted through conjugation to the aromatic protons and, to a smaller extent, to the NMe_2 group, which lies almost outside the cavity. The methyl protons at the other extreme of the dye molecule are similarly unaffected by the CD microenvironment, since they also lie practically outside the host cavity.

We had previously suggested[9] that a comparison of the theoretical energies obtained from the application of the LIE method to different β-CD/dye complexes could be used to predict relative stabilities of various

Table 3. Calculated interaction energies for complexes between dyes **1**, **2**, and **3** in various orientations and cyclodextrins ΔE^{total}

Dye–CD Complex	Calculated energy values (kcal·mol^{-1})[a]				
	$\Delta E^{\text{non-polar}}_{\text{free}}$	$\Delta E^{\text{non-polar}}_{\text{bound}}$	$\Delta E^{\text{polar}}_{\text{free}}$	$\Delta E^{\text{polar}}_{\text{bound}}$	ΔE^{total}
1[b]	-20.73 ± 3.60	-30.17 ± 1.68	-20.15 ± 6.61	-7.14 ± 1.69	3.57
2a[c]	-36.24 ± 3.61	-40.34 ± 2.04	-30.28 ± 5.87	-9.47 ± 1.96	16.71
2b[d]	-36.24 ± 3.61	-41.89 ± 2.76	-30.28 ± 5.87	-6.66 ± 2.80	17.97
3[e]	-22.00 ± 2.77	-27.36 ± 2.18	-20.79 ± 4.87	-4.80 ± 1.74	10.63

[a] For a definition of the energy terms, see the text and Eqns (5) and (6).
[b] Complex with β-CD, see Ref. 9.
[c] Complex with β-CD, with the aromatic phenyl ring inside the host cavity, see Ref. 9.
[d] Complex with β-CD, with the aromatic phenyl ring outside the host cavity, see Ref. 9.
[e] Complex with γ-CD.

conformations, in spite of the fact that these energies might deviate significantly from experimental binding energies. A comparison of the energy values ΔE^{total} of Table 3 with the experimental association constants K of Table 1 suggests some qualitative correlation between these terms. ΔE^{total} values increase in the order β-CD/**1** $< \gamma$-CD/**3** $< \beta$-CD/**2**. As argued before,[9] complex stability should be greatest for the most negative, or least positive ΔE^{total} value. Thus, stability should decrease in the order β-CD/**1** $> \gamma$-CD/**3** $> \beta$-CD/**2**, in agreement with the K values listed in Table 1, $1001 > 447 > 138\,\text{M}^{-1}$, respectively.

In this analysis, we are comparing complexes with different CDs. Any conclusions drawn from such a small set of examples must be taken with caution. We cannot decide at this stage if the trend observed for the above complexes may be extrapolated to CD inclusion complexes in general. If this is true, energy values from simulations employing the LIE method could be used to estimate binding energies for any CD inclusion complex, in which case the α and β parameters of Eqn (5) should not vary for different host or guest molecules. In order to verify these hypotheses, a larger set of experimental

association constants K and theoretical energy values from the LIE method should be available, covering a wider variety of CD complexes. Further work in this line is under way in our laboratories.

EXPERIMENTAL

UV–visible spectra were recorded with a Unicam UV4 spectrophotometer. ^{1}H-NMR spectra of the pure compounds were obtained with a Bruker Avance 400-MHz equipment, employing tetramethylsilane as internal standard. Melting points were recorded with a Micro-thermal capillary melting point apparatus and were not corrected.

α-, β-, and γ-CDs were purchased from Aldrich. Dye **1** was prepared by condensation of 4-(dimethylamino)ben-zaldehyde with N,N-dimethylbarbituric acid (Aldrich), as described previously,[12] mp 225–227°C, lit.[12] mp 224–226°C ^{1}H-NMR (CDCl$_3$) δ 3.16 (s, 6H, NMe$_2$); 3.40 (s, 6H, CON—CH$_3$); 6.70 (d, 2H, J = 9 Hz, ArH *ortho* to NMe$_2$); 8.41 (d, 2 H, J = 9 Hz, Ar-H *meta* to NMe$_2$); 8.44 (s, 1 H, C=CH). Dye **2** was prepared in a similar way, by condensation of N,N-dimethylbarbi-turic acid with 4,4'-*bis*(dimethylamino)benzophenone, mp 226–228°C, lit.[12] mp 227–230°C. ^{1}H-NMR (CDCl$_3$) δ 3.13 (s, 12H, N(CH$_3$)$_2$); 3.33 (s, 6H, CON—CH$_3$); 6.65 (d, 4H, J = 9 Hz, ArH *ortho* to NMe$_2$); 7.25 (d, 4H, J = 9 Hz, Ar-H *meta* to NMe$_2$). Compound **3** was obtained by condensation of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (Aldrich) with 4-(dimethylamino) benzaldehyde following our reported procedure[13], mp 167–169°C, lit.[13] mp 165–167°C. ^{1}H-NMR (CDCl$_3$) δ 1.74 (s, 6H, OC(CH$_3$)$_2$); 3.13 (s, 6H, N(CH$_3$)$_2$); 6.66 (d, 2H, J = 9.5 Hz, Ar-H *ortho* to NMe$_2$); 8.22 (d, 2H, J = 9.5 Hz, Ar-H *meta* to NMe$_2$; 8.27 (s, 1H, C=CH).

Aqueous solutions of dyes **1** and **3** were prepared with distilled water, to which 5% v/v of methanol was added. Aqueous solutions of dyes **2** required no added methanol for solubilization. In all cases, sonication was employed to facilitate the solubilization of the dyes in the aqueous media. Solutions for the ^{1}H-NMR spectra of dyes **2** and **3**
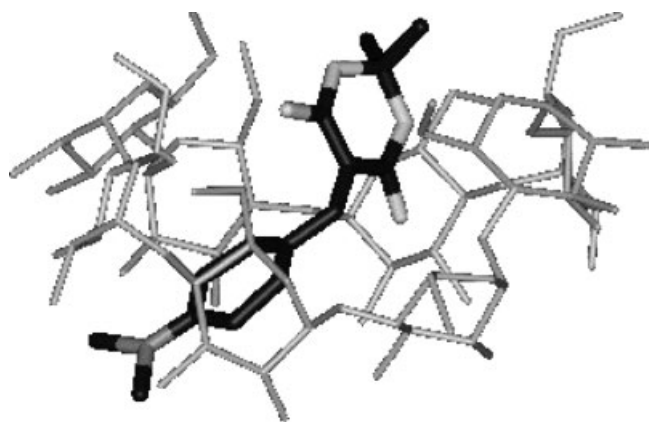

Figure 6. Structure of the dye **3**/γ-CD complex in water, after molecular dynamics simulations. For the sake of clarity, all water molecules and hydrogen atoms are omitted

in the presence of CDs were prepared in 1:1 vol mixtures of D_2O:CD_3OD (99+ atom %, Aldrich). The residual solvent signal for CD_3OD (δ 3.31 referred to TMS) was employed as internal reference for all observed chemical shifts in these mixtures.

Molecular dynamics simulations were performed with the CHARMM27 force field[14] employing the crystallized γ-CD structure available from the PDB crystallographic data base.[15] The structure of dye **3** was generated with InsightII[16] and optimized with the AM1 basis set. The partial atomic charges of the molecules were calculated using the restrained electrostatic potential (RESP) fitting procedure. Electrostatic potentials were generated at the Hartree–Fock /6-31G* level. The dye was docked into the γ-CD cavity using Autodock v 3.0[17] and the most stable conformation chosen as starting point for simulations in water. Then two simulations were performed in water, one involving the dye and the other the dye/γ-CD complex. The molecule and complex were soaked into a 20-Å-radius sphere built with the TIP3P water model.[18] After an initial minimization, followed by a 600-step heating to 300 K, the system was allowed to reach equilibrium through 500 steps, followed by an acquisition period of 200 ps. During data collection, all systems showed potential-energy fluctuations smaller than 10%. All calculations were done using a cutoff value of 10 Å.

Acknowledgements

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