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A Spectral and Molecular Dynamics Simulation Study of β-Cyclodextrin Inclusion Complexes with Solvatochromic Dyes Derived from Barbituric Acid

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Abstract

The linear interaction energy (LIE) method was applied to the molecular-dynamics-simulation study of the interactions in water between β-cyclodextrin and two solvatochromic dyes derived from *N*,*N*-dimethylbarbituric acid, the 5-[(4-dimethylaminophenyl)methylene]- (**1**), and the 5-[bis(4-dimethylaminophenyl) methylene]-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione (**2**). Both compounds interact with β-CD by insertion of an aminophenyl ring into its hydrophobic cavity. Theoretical and spectral evidences point to a greater association of compound (**1**) than dye (**2**) with cyclodextrin in water.

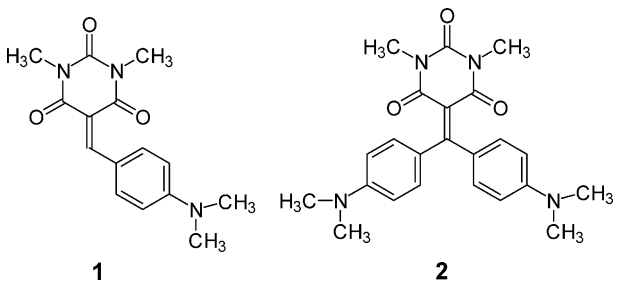
Introduction

Cyclodextrin (CD) inclusion complexes have been widely studied in the past decades [1, 2]. Evidences for the formation of these complexes have been obtained with a variety of analytical techniques that include optical [3–6], electrochemical [3, 5–7], diffusion measurements [8–10] and NMR spectral methods [10–13]. The mode of insertion of organic guests into the cyclodextrin cavity has been investigated by molecular mechanics and dynamics simulations [4, 13–16].

One reason for this interest stems from the fact that aqueous solutions of cyclodextrins provide a convenient model for a micro heterogeneous environment where an external, polar bulk phase coexists with a less polar medium inside the cyclodextrin cavity. Unlike micelles and other microheterogeneous systems, formed by the association of surface-active compounds in water, the cyclodextrin molecule is a comparatively small system, with a defined geometry, offering a clearer picture for the study of host-guest interactions.

The inclusion of dyes into the cyclodextrin cavity may lead to changes in the spectra of their aqueous solutions [17, 18]. Such behavior is an indication of changes in the solvation of these dyes. We may thus design solvatochromic compounds where the donor and acceptor fragments interact differently with the non-polar cyclodextrin cavity, leading to the preferential insertion of one moiety, and exposing the other fragment to the bulk aqueous solution. We could thus exploit this system to study the effect of the microenvironment around different parts of the dye. A few recent papers have addressed the interesting question of the orientation of a solvatochromic dye in the interface of a heterogeneous system [19–21]. The reported evidence of two different spectral features for the same dye was interpreted as arising from different orientations of the chromophore in the interface [21]. This subtle effect was thus capable of detecting changes in the microenvironment of different parts of a solvatochromic dye.

Following this line of reasoning, we decided to exploit the possible formation of inclusion complexes with cyclodextrins to investigate the effect of environmental changes on a donor or on an acceptor fragment of a solvatochromic dye. We had recently described the solvatochromic properties of dyes **1** and **2**, where a donor N,N-dimethylaminophenyl group transfers charge to an acceptor barbituric ring system [22, 23].



Scheme 1.

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In the present communication we studied the interactions of these dyes with β-cyclodextrin, by analysis of their UV–vis spectra coupled with molecular dynamics simulations of the 1:1 CD complexes in solution. Host-guest structures differing in the dye orientation were compared by means of an interaction energy method, where all simulations were performed in explicit water.

Experimental

UV-visible spectra were recorded with a UNICAM UV4 Spectrometer.

β-Cyclodextrin was purchased from Aldrich. Compounds **1** and **2** were prepared as described previously [22].

Aqueous solutions of dye **1** were prepared with distilled water, to which 5% v/v of methanol was added, because of the poor solubility of these compounds in pure H_2O. Aqueous solutions of dye **2** required no added methanol for solubilization.

Molecular dynamics simulations were performed with the CHARMM27 force field [24] employing the crystallized β-CD structure available from the Cambridge crystallographic data base [25]. Dyes **1** and **2** were generated with InsightII [26] 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. Dyes were docked into the β-CD cavity using Autodock v 3.0 [27] and the most stable conformations chosen as starting point for simulations in water. For molecules **1** and **2**, two simulations were performed in water, one involving the dye and the other the dye/β-CD complex. The molecules and complexes were soaked into a 20-Å-radius sphere built with the TIP3P water model [28]. The same protocol was followed for all dynamics. It consisted of an initial minimization, followed by a 300-step heating to 300 K, an equilibration of 500 steps and 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.

Results and discussion

Spectral variations of dyes **1** and **2** in water, in the presence of increasing concentrations of β-cyclodextrin, are shown in Figures 1 and 2.

Formation of inclusion complexes with β-CD was evident for dye **1** (Figure 1). The situation with dye **2** was less clear, since the observed spectral variations were not significant after a nearly 10-fold increase in the concentration of added β-cyclodextrin. (Figure 2)

The spectrum of dye **1** exhibited a hyperchromic shift of its longest-wavelength band upon addition of

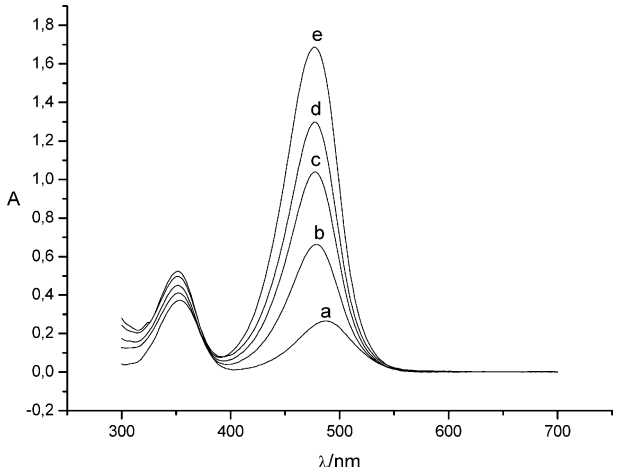


Figure 1. Variation of the longest-wavelength charge-transfer band of dye **1** in water ($c = 5.22 \times 10^{-6}$ mol dm^{-3}) in the presence of increasing concentrations of β-cyclodextrin [β-CD] = 0 (a); 3.25×10^{-3} (b); 7.40×10^{-3} (c); 1.03×10^{-2} (d) and 2.11×10^{-2} mol dm^{-3} (e).

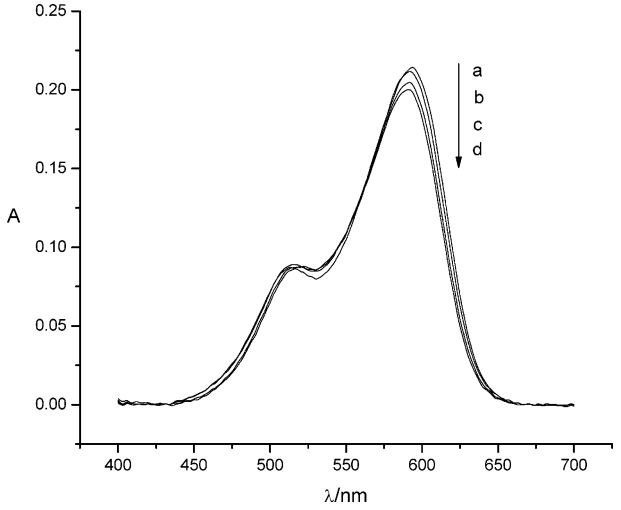


Figure 2. Variation of the longest-wavelength charge-transfer band of dye **2** in water ($c = 5.9 \times 10^{-6}$ mol dm^{-3}) in the presence of increasing concentrations of β-cyclodextrin. [β-CD] = 0 (a); 3.0×10^{-3} (b); 10.5×10^{-3} (c) and 22.0×10^{-3} mol dm^{-3} (d).

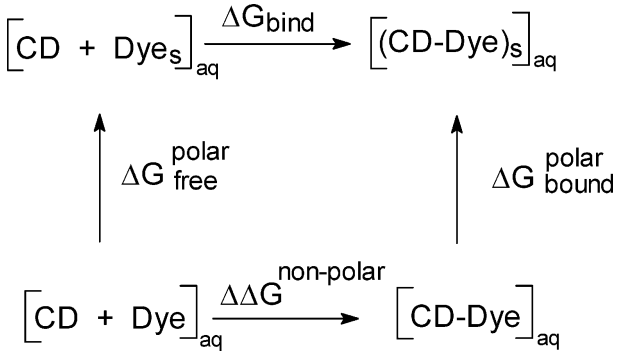
increasing concentrations of β-CD. This was accompanied by a slight decrease of its λ_{max} value, as can be seen in Figure 1. These spectral changes may be interpreted as arising from environmental changes experienced by the dye. The shifts indicate that a less polar environment gradually replaces the aqueous milieu surrounding the dye, as the cyclodextrin host is added to the solution.

The determination of the binding constants for these systems by the analysis of their spectra in solution was rendered difficult by the variations in both intensity and position of the maxima with increasing concentrations of cyclodextrin. In addition, in all cases, we could not attain complete dye association, because of solubility problems. Nevertheless, deconvolution analysis of the obtained spectra led to an estimated value of 1001 ± 84 M^{-1} for the 1:1 association constants of dye **1** with β-CD.

In order to obtain a more detailed picture of the interactions between dyes **1** and **2** with the cyclodextrins, we carried out molecular dynamics simulations for the formation of inclusion complexes of these compounds with β-CD in water.

Various examples of simulation applied to cyclodextrin complexes are found in the literature [4, 13–16, 29, 30]. Although energy balances for host-guest complexations have been successfully estimated by treating the solvent as a continuum [16, 31], it has been observed that a simulation without an explicit treatment of the solvent gives unreliable results [14].

We followed the approach developed by Åqvist *et al.* [32], relying on the linear interaction energy (LIE) method. Attractive features of this method include its simplicity and the fact that all simulations are performed in explicit water, yielding a more realistic picture of solute-solvent interactions than the continuum model. The method is based on the application of a thermodynamic cycle, which is depicted below for our particular case.



Scheme 2.

The subscript *s* characterizes a solvated species, with electrostatic interactions with its surrounding "turned on" in the free and bound states. The binding free energy ΔG_{bind} is the sum of a non-polar contribution $\Delta\Delta G^{non\text{-}polar}$ and a polar contribution $\Delta\Delta G^{polar} = \Delta G^{polar}{}_{bound} - \Delta G^{polar}{}_{free}$. Binding free energies are obtained by correcting the calculated energy variations by means of calibration factors α and β, obtained by fitting equation (1) with a set of experimental values.

$$\Delta G_{bind} = \alpha(\Delta E^{non\text{-}polar}_{bound} - \Delta E^{non\text{-}polar}_{free}) + \beta \cdot (\Delta E^{polar}_{bound} - \Delta E^{polar}_{free}). \quad (1)$$

The calibration factor α corrects non-polar, whereas β affects polar energy variations. Values of α and β in the range of 0.14–0.82 are found in the literature, depending on the system under study [32–34].

In order to calculate ΔG_{bind} values for the CD complexes under study, we needed to estimate α and β values for our system. Although host-guest binding energies for cyclodextrin complexes could be obtained from the literature, their corresponding simulation energies by the LIE method were not available. Thus, the lack of reliable values of α and β for CD-guest complexes precluded the calculation of binding energies for our systems.

This problem was circumvented by considering that, for the purpose of comparing different structures, we did not need to compute the absolute values of their binding energies, but only the corresponding sum of the calculated electrostatic ($\Delta E^{polar}{}_{bound} - \Delta E^{polar}{}_{free}$) and van der Waals ($\Delta E^{non\text{-}polar}{}_{bound} - \Delta E^{non\text{-}polar}{}_{free}$) contributions.

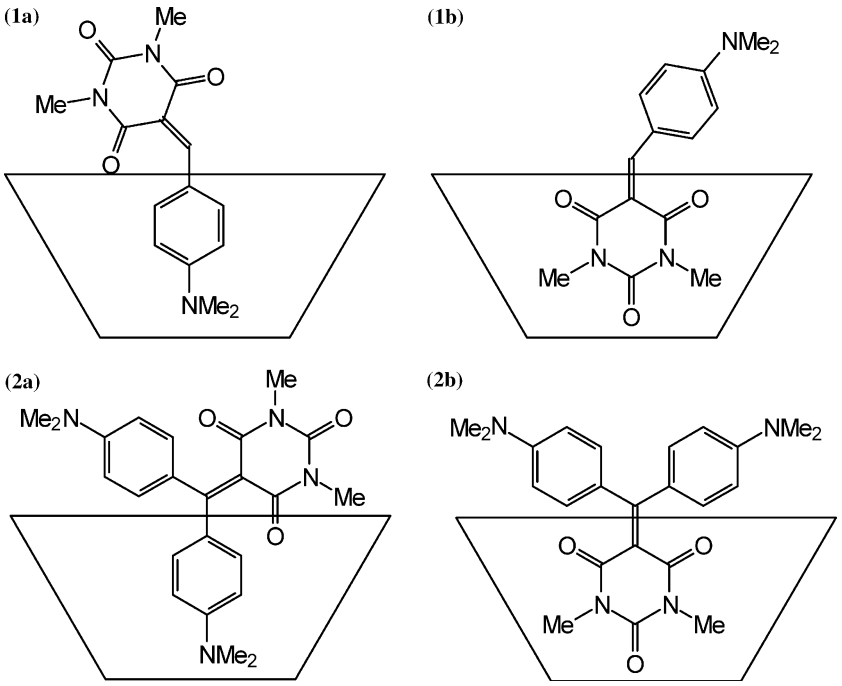
Our treatment was based on the following analysis of equation (1). In general, electrostatics disfavor host-guest binding ($|\Delta E^{polar}{}_{bound}| < |\Delta E^{polar}{}_{free}|$). The main driving force for complexation are non-bonded van der Waals interactions between the host and the guest in water [16]. Since α and β are positive, we may assume that, whatever the value of β in equation (1), the polar contribution β ($\Delta E^{polar}{}_{bound} - \Delta E^{polar}{}_{free}$) will be positive, thus opposing and reducing the overall negative binding free energy ΔG_{bind}. The non-polar term, α ($\Delta E^{non\text{-}polar}{}_{bound} - \Delta E^{non\text{-}polar}{}_{free}$), whatever the value of α, will have to be negative, and larger than the polar term, to compensate for the unfavorable contribution of the latter. Thus, although we do not have optimum values for α and β, we may assume that, for a pair of complexes with alternative orientations, the one with the largest, negative (or the smallest, positive) sum ΔE^{total} of the electrostatic ($\Delta E^{polar}{}_{bound} - \Delta E^{polar}{}_{free}$) and van der Waals ($\Delta E^{non\text{-}polar}{}_{bound} - \Delta E^{non\text{-}polar}{}_{free}$) contributions will be the most stable. Of course, this sum ΔE^{total} cannot be taken as the theoretical binding energy of the dye-CD complex in water, but is simply used as a qualitative criterion in comparing alternative orientations.

Following this criterion, we calculated the non-polar and polar contributions of the free and bound dyes **1** and **2**, starting from variously docked dye-CD complexes. For each system we performed two simulations, corresponding to the two vertical legs of the thermodynamic cycle depicted above. Each simulation yielded a polar (ΔE^{polar}) and a non-polar ($\Delta E^{non\text{-}polar}$) contribution to the total energy.

For compound **1** two different orientations of the dye were considered as starting points for the dynamics in water. In one case (complex **1a**) the aminophenyl ring was docked inside the CD cavity; in the other case (complex **1b**), this situation was reversed and the barbituric ring system was docked inside the host cavity. For compound **2** we also considered two possibilities, inserting the aminophenyl group into the cavity in one case (complex **2a**), or the barbituric ring system in the other (complex **2b**).

The calculated energy variations for each system are given in Table 1.

The results of Table 1 indicate that, for dye **1,** the energy balance clearly favors structure **1a**, in agreement with the expectations that the more polar barbituric ring system should be solvated by the aqueous environment



Scheme 3.

Table 1. Calculated interaction energies between dyes **1** and **2**, in various orientations, and β-cyclodextrin

	Energy values (kcal mol^{-1})				
Dye-CD Complex	$\Delta E^{\text{non-polar}}{}_{\text{free}}$	$\Delta E^{\text{non-polar}}{}_{\text{bound}}$	$\Delta E^{\text{polar}}{}_{\text{free}}$	$\Delta E^{\text{polar}}{}_{\text{bound}}$	ΔE^{total}
1a	−20.73 ± 3.60	−30.17 ± 1.68	−20.15 ± 6.61	−7.14 ± 1.69	3.57
1b	−20.73 ± 3.60	−21.23 ± 2.22	−20.15 ± 6.61	−3.84 ± 2.06	15.31
2a	−36.24 ± 3.61	−40.34 ± 2.04	−30.28 ± 5.87	−9.47 ± 1.96	16.71
2b	−36.24 ± 3.61	−41.89 ± 2.76	−30.28 ± 5.87	−6.66 ± 2.80	17.97

outside the CD cavity. Solvation takes place through hydrogen bonds between the carbonyl oxygen atoms of the guest and the hydroxyl groups of the outer CD rim and of surrounding water molecules (Figure 3). One carbonyl oxygen of the barbituric ring, for example, binds with a neighboring hydroxyl group of the host cyclodextrin, with a distance of O · · · · H 1.87 Å. By contrast, when the barbituric moiety is inside the hydrophobic cavity (structure **1b**), the CD hydroxyl proton nearest to its carbonyl oxygens is 3.9Å away from it. In addition, the less hydrophilic aminophenyl group reinforces the preference for structure **1a**, by its insertion into the hydrophobic CD cavity.

For the CD-dye **2** complex, both structures possess similar stabilities in water. The values of Table 1 indicate that this arises from similar non-polar contributions in the two structures. Apparently the conformations of the barbituric and the aryl ring systems vis-à-vis the CD host are important here. Discrimination between the two ring systems by the external aqueous environment is greater when either of them protrudes vertically outside the CD cavity, as in complex **1a**, than when they both lie flat, acting as a convex lid which covers the β-CD opening, as in structure **2a** (Figure 4). As a result, there is little difference between the alternatives of a barbituric (structure **2a**) or an aryl ring (structure **2b**) outside the CD cavity, though the former is still slightly more stable.

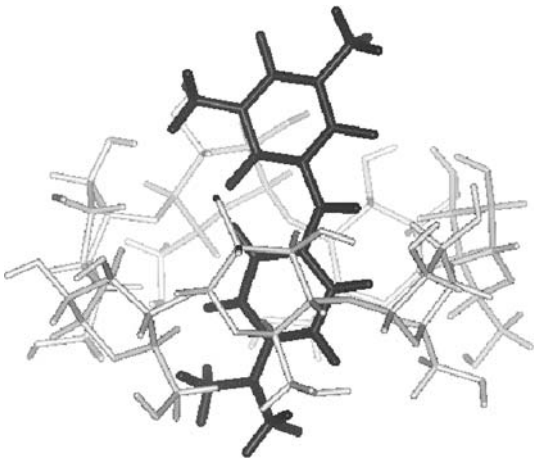


Figure 3. β-CD/Dye **1** complex in water (structure **1a**). For the sake of clarity, the solvating water molecules are not shown. Dye **1** is shown in black, the CD host in grey.

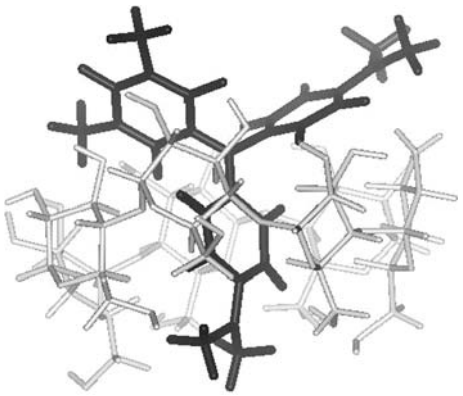


Figure 4. β-CD/Dye **2** complex in water (structure **2a**). For the sake of clarity, the solvating water molecules are not shown. Dye **2** is shown in black, the CD host in grey.

The data of Table 1 also point to a larger binding energy between β-CD and dye **1** than with dye **2**. This is confirmed by the spectral variations shown in Figures 1 and 2, where a nearly 10-fold increase in the concentration of the host molecule induces more significant changes in the spectra of **1** than of **2**.

In conclusion, spectral variations of solutions of dyes **1** and **2** in the presence of increasing concentrations of β-cyclodextrin pointed in both cases to the formation of 1:1 CD-dye complexes in water. An association constant of $1001 \pm 84\ M^{-1}$ was estimated for dye **1**. Solubility problems rendered more difficult the determination of a constant for dye **2,** though the observed spectral variations suggested a smaller binding interaction between the dye and the cyclodextrin molecule. Molecular simulations based on the LIE method confirmed these observations, indicating in addition that the preferred orientation for the β-CD/dye **1** complex should correspond to structure **1a**, with insertion of the aryl ring into the CD cavity. In the case of dye **2**, the alternative structures **2a** and **2b** should exhibit similar stabilities, with a slight preference for the aryl ring insertion into the hydrophobic cyclodextrin cavity. The above analysis thus showed that the LIE method may be used as a valid theoretical tool for studying host-guest interactions in aqueous solutions, even when a set of calculated energy data for a family of compounds is not available for estimation of reliable values for the α and β parameters.

Acknowledgement

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