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ORIGINAL ARTICLE

Application of automated docking to the binding of naphthalenes to βCD in water: correlation with spectrofluorimetric data

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Abstract Automated semi-rigid docking has been explored as an alternative approach for the theoretical study of the inclusion complexes with cyclodextrins. To this purpose we have chosen as a model for the binding to βCD some naphthalene derivatives (naphthalene, 2-ethylnaphthalene, 2-acetylnaphthalene, 1-naphthyl acetate, 2-naphthyl acetate and 1-naphthol). For comparison purposes, the binding constants in water and the associated thermodynamic parameters have been obtained under the same experimental conditions by steady-state fluorescence spectroscopy. The calculations of the automated docking regarding the topology of the guest inside the cavity produce a cluster of structures that qualitatively agrees with fluorescence results and literature data. However, the predicted values of the free energy of binding are lower than the experimental ones by ca. –10 kJ mol^{-1}, and very close to the experimental enthalpy of binding deduced from the temperature dependence of the association constants. The differences are ascribed mainly to the assumption of rigidity of the CD into the auto-docking scheme.

Keywords Cyclodextrins · Inclusion complexes · Molecular modeling · Naphthalene derivatives · Semi-rigid docking

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Introduction

Computational Chemistry has been widely utilized in the cyclodextrin field for the prediction of the geometry of the complexes and the estimation of binding energies [1]. Yet, the relatively large size of these macrocycles and the key role that the solvent plays in the complex formation force to introduce assumptions and restrictions, which make the results often unreliable. There are some investigations in the literature dealing with molecular modeling applied to the complexation of naphthalenes with cyclodextrins. Many of these investigations complement spectroscopic studies such as fluorescence [2, 3] or RMN ones [4, 5], which make use of different strategies, such as molecular dynamics or rigid docking. These compounds are considered as model guests for the study of the inclusion processes due to their hydrophobicity, spectral properties, and possibility of chemical substitution.

The aim of this work has been to test a less explored methodology of molecular mechanics, namely automated semi-rigid docking, by considering the binding of some naphthalene derivatives (naphthalene, 2-ethylnaphthalene, 2-acetylnaphthalene, 1-naphthyl acetate, 2-naphthyl acetate and 1-naphthol) to βCD. The calculations have been carried out with AutoDock 3.0.5 software, a program intended for predicting the interaction of ligands with biomacromolecular targets by an automated procedure [6, 7, 8]. In our case, the CD acts as the rigid receptor, whereas torsions are allowed in the guest (ligand). Starting from an arbitrary configuration of the guest inside the CD, the program uses a Lamarckian genetic algorithm for searching the most favorable orientations, leading to a cluster of structures of favorable energies. The program also

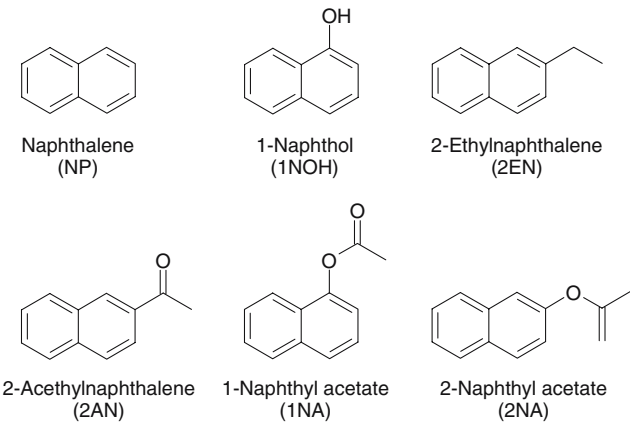
provides the free energy of binding by calculating the energies of host, guest and complex in vacuum, and their energies of solvation. The free energy is modeled semiempirically by introducing entropic terms into the molecular mechanics equations. This contribution accounts for the restriction of internal rotors and global rotation and translation, and also the desolvation upon binding and the hydrophobic effect [9].

In order to reach safe conclusions from the theoretical calculations, we have measured the binding constants and associated thermodynamic parameters of the different guests under the same experimental conditions by steady-state fluorescence spectroscopy, the most appropriate technique due to the high quantum yield and poor solubility of these compounds. This has been necessary due to either the inconsistency in the literature data coming from different experiments, or the lack of thermodynamic parameters. In this way, this work also revises the existing data about the inclusion complexes of naphthalenes with βCD.

Experimental methods

Chemicals

Naphthalene derivatives (Scheme 1) were obtained from Aldrich (naphthalene, NP, 2-ethylnaphthalene, 2EN, and 2-acetylnaphthalene, 2AN), Acros Organics (1-naphthyl acetate, 1NA, and 2-naphthyl acetate, 2NA) and Merck (1-naphthol, 1NOH). β-cyclodextrin (11.10% water content) was kindly donated by Roquette (Laisa España S.A.). All the chemicals (purity higher than 99%, except 2-naphthyl acetate with 98%) were used as received. Solutions were prepared with freshly deionized water.



Scheme 1

Fluorescence measurements

Steady-state fluorescence spectra were recorded with an LS–50B Perkin-Elmer spectrofluorimeter. Data acquisition and analysis of emission spectra were performed with the Fluorescence Data Manager Software supported by Perkin-Elmer. The cell housing (1.000-cm path cells, quartz cuvette) was controlled at the temperature of interest (15, 20, 25, 30, 35 and 40°C) with a precision of ±0.1°C using a Lauda Ecoline RE104 thermostat connected to the instrument. The excitation was at the absorption maximum in each case, with typical excitation and emission slits of 4–6 nm. The concentration of guest (naphthalene derivative) was kept constant at 5.88×10^{-6} mol l^{-1}, while varying that of βCD up to 4.05×10^{-3} mol l^{-1} in constant increments added with a micropipette on the cell.

Computational studies and methodology

The construction and structure refining of the host and guest molecules were performed with Insight II software on an SGI Octane2 workstation, employing the CVFF forcefield [10]. For the energy minimization we used the different algorithms supplied with the Discover module successively (steepest descents, conjugate gradients and Newton-Raphson) until the root-mean squares of the derivatives were less than 0.0001 kcal $Å^{-1}$. The naphthalenes have been docked to the βCD with AutoDock 3.0.5 [9]. In order to compute the interaction energies, AutoDock generates three-dimensional grids, one for each atom type present in the ligand (C, O, H), where each point within the grid stores the potential energy of a probe atom due to all the atoms of the macrocycle. Then, at every point, the pairwise interaction energy between host and guest is derived from 12,6-Lennard-Jones potentials for van der Waals forces, and Coulomb for electrostatic interactions. Partial charges of the molecules for the automated docking were taken from the CVFF forcefield. In our calculations, the grid positions were 0.375 Å apart in a 22.5 Å cubic box centered at the CD cavity. For the search strategy we used the genetic algorithm search method provided with AutoDock, with 256 runs and 100 structures per run. The effect of the solvent is taken into account by generating atomic solvation parameters for the molecules.

Results and discussion

Fluorescence measurements

The fluorescence of all the naphthalene derivatives changes in the presence of βCD. There is an intensity

enhancement upon addition of CD, with the sole exception of AN, which undergoes quenching of its emission. Fig. 1a, b show the emission spectra of NP and 2AN, respectively, in aqueous solution in the presence of increasing βCD concentrations, as examples of the two different trends in the fluorescence intensity. The F_0/F plots show the usual pattern for CD complexes: increasing the temperature reduces the stability of the complex, as corresponds to an exothermic process (Fig. 2). In the case of a 1:1 binding, in which both the complex and the substrate are fluorescent, the change in intensity is given by

$$\frac{F_0}{F} = \frac{1 + K[\mathrm{CD}]}{1 + aK[\mathrm{CD}]}$$

F_0 being the fluorescence of the guest, F the measured intensity at each point, [CD] the concentration of free βCD, and K the binding constant; a is related to the quantum yields and absorptivities of the guest in its free and complexed form through $a = \varepsilon_{cx}\phi_{cx}/\varepsilon_{guest}\phi_{guest}$. Both parameters were calculated by a nonlinear least-squares regression analysis based upon this equation [11]. From the dependence of K with the temperature, it is possible to deduce the enthalpy and entropy of the binding process using the van't Hoff equation. The fitted curves of NP have been included as solid lines in Fig. 2, and the resulting parameters at the different temperatures are collected in Table 1.

As can be inferred from Table 1, the derivatives in position 2 present similar constants to those of the NP:βCD complex, in accordance with literature data [12], although they are somewhat higher for 2EN and 2NA. On the other hand, the constant for 1NA is almost 3.5 times lower than that of its 2-position isomer. This result agrees with the ratio of binding constants proposed by Barros et al. (among 3.2 and 4.2) [12]. In contrast, 1NOH presents the highest affinity for the βCD. This K value is close to the bibliographic one and the small size of its substituent could be the reason of the tighter binding, despite this derivative has the substituent in position 1 [13].

For all the systems the inclusion enthalpy and entropy are negative. Some of the values for the entropy differ from the literature results. For example, Fraiji et al. [14] found a positive change in entropy for the complex of 2AN with βCD. In general, negative as well as positive values of entropy for naphthalene derivatives have been usually found [15, 16]. Regarding the absolute values of the enthalpy, they range from –18 for NP to –37 kJ mol^{-1} in 1NA. This last value is somewhat uncertain because of the weak temperature dependence of K in this case (we could not obtain reliable values of the binding constant at some temperatures, left as a blank in Table 1).

Automated semi-rigid docking

The results of the docking are shown in Figs. 3, 4. For the sake of clarity, only some of the most stable conformations of the cluster of structures have been included in the picture. NP and 1NOH, the least bulky guests, penetrate deeply in the cavity, in an equatorial form, as Harata et al. found for the derivatives in 1-position [17]. The same equatorial orientation was

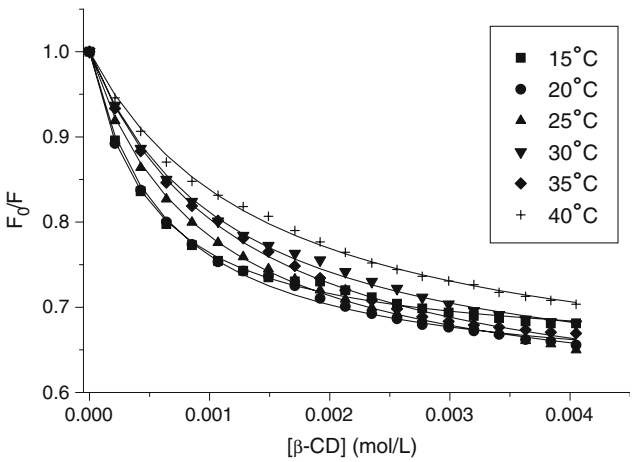


Fig. 2 F_0/F ratio for naphthalene in aqueous solution at different temperatures. The nonlinear fit is shown for each plot (solid lines)

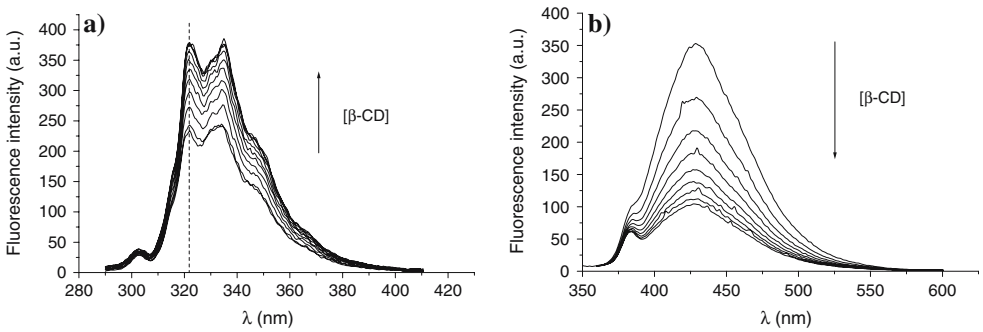


Fig. 1 Emission spectra of naphthalene (**a**), and 2-acetylnapyhalene (**b**) in aqueous solutions in the presence of increasing βCD concentrations

Table 1 Binding constants and thermodynamic parameters for the complexes of naphthalene derivatives with βCD

	K (M^{-1})						$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})
	15°C	20°C	25°C	30°C	35°C	40°C		
NP	–	640	608	517	453	405	–18 ± 2	–9 ± 5
2EN	1172	765	752	725	565	427	–26 ± 4	–30 ± 10
2AN	649	569	491	460	400	329	–20 ± 1	–14 ± 4
1NA	463	–	252	–	–	134	–37 ± 2	–78 ± 6
2NA	1179	950	926	786	620	568	–22 ± 2	–17 ± 7
1NOH	1439	1104	1014	778	672	649	–25 ± 2	–26 ± 8

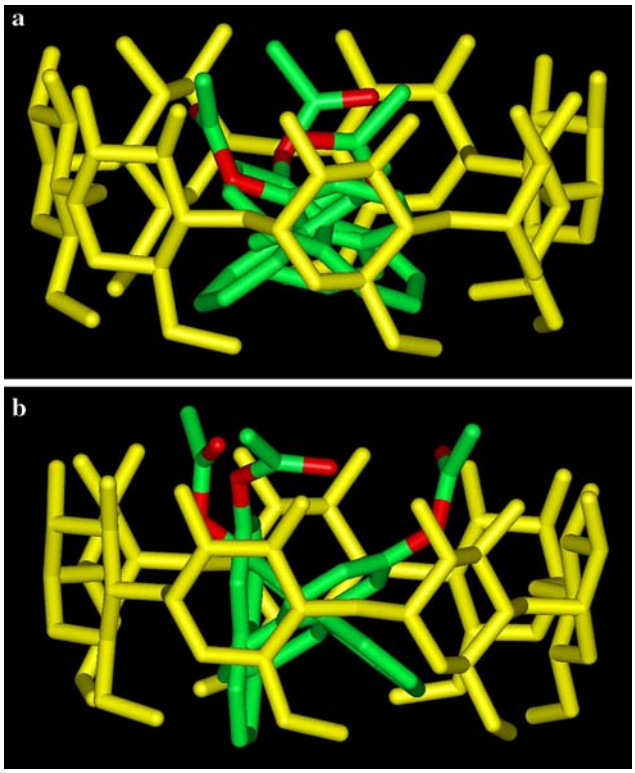


Fig. 3 Structures of (**a**) 1-naphthyl acetate and (**b**) 2-naphthyl acetate complexes with βCD (substituent towards secondary hydroxyls)

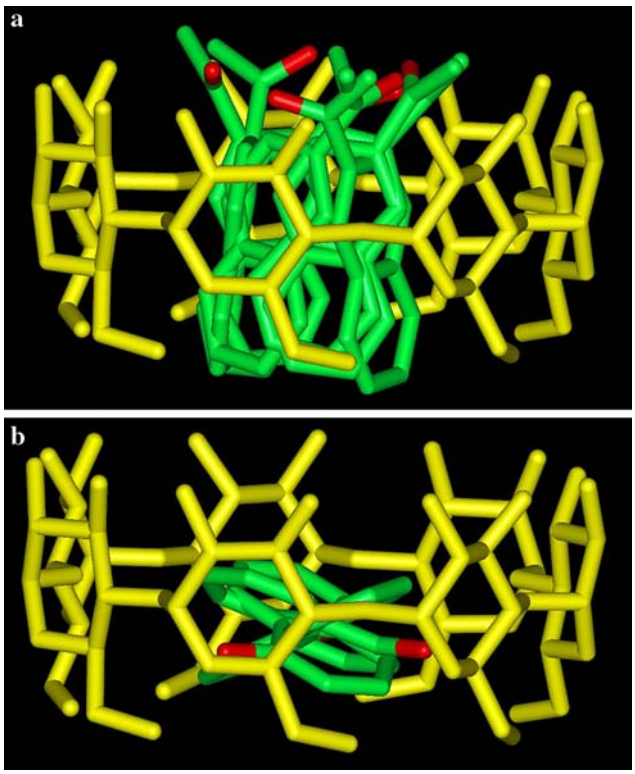


Fig. 4 Structures of the complexes of βCD with (**a**) 2-acetylnaphthalene; (**b**) 1-naphthol

expected in principle for 1NA, according to the Harata's rule, but the size of the substituent does not allow a whole equatorial inclusion, so it enters the cavity axially, more tilted with respect to the 2-isomer (Fig. 3). Two-substituted naphthalene derivatives display an axial orientation as shown in Fig. 4 for 2AN. For all the derivatives, the most stable structures have the substituent oriented towards the secondary hydroxyls (the wider rim of the CD) for most of the conformations. We have designated as n_1 in Table 2 the fraction of conformations with this orientation, and as n_2 the fraction of structures with substituent towards the primary border.

The free energies of binding for the different derivatives obtained by the semi-rigid approach are very similar. The lowest value is obtained for 1NA (–28.83 kJ mol^{-1}) and the highest for NP (–25.76 kJ mol^{-1}), whereas the rest of the derivatives have practically the same energies. These values are systematically ca. –10 kJ mol^{-1} lower than the experimental ones, which are also very similar to each other. Surprisingly, the experimental enthalpy of inclusion roughly matches the computed free energies of binding. The rigidity of the receptor, which is taken for granted in the philosophy of AutoDock for proteins, might be a drastic assumption in the case of a cyclodextrin. The presence of the naphthalene inside the cavity must

Table 2 Results of conformations and interaction energies for the naphthalene derivatives with βCD obtained by semi-rigid docking at 298 K. The last column includes the experimental ΔG°_{298}

	n_1	n_2	ΔG°_1 (kJ mol^{-1})	ΔG°_2 (kJ mol^{-1})	ΔG°_{av} (kJ mol^{-1})	ΔG°_{298} (kJ mol^{-1})
NP	0.99	0.01	–25.76	–25.46	–25.76	–15.8
2EN	0.83	0.17	–26.91	–26.23	–26.79	–16.4
2AN	0.98	0.02	–27.29	–24.96	–27.25	–15.4
1NA	0.96	0.04	–28.90	–27.23	–28.83	–13.9
2NA	1	0	–28.27	–	–28.27	–16.7
1NOH	1	0	–27.69	–	–27.69	–17.0

n_1: fraction of molecules with the substituent towards the secondary rim

n_2: fraction of molecules with the substituent towards the primary rim

distort in a certain extent the high symmetry of the CD, which implies changes in the entropy and enthalpy that may bias the results. A possible way of overcoming this drawback, keeping the simplicity of the semi-rigid docking scheme, could be to develop a more convenient free energy function, calibrated with the copious data available from binding constants of inclusion complexes of cyclodextrins.

Conclusions

Automated semi-rigid docking has been applied to the study of the inclusion of some naphthalene derivatives into βCD, and the binding constants, inclusion enthalpy and entropy have been calculated from the analysis of the fluorescence data. The cyclodextrin complexes show higher fluorescence intensities than the guest except in the case of 2-acetylnaphthalene where the emission is quenched upon the complex formation. Molecular modeling calculations via semi-rigid docking qualitatively agree with the fluorescence results, indicating a preferential orientation of the guest inside the cavity. Isomers in position two penetrate the CD axially, leaving part of its substituent outside the CD, while naphthalene and 1-naphthol are lodged in an equatorial orientation. Although the computed values of the free energies of binding are similar for the guests under study, they differ by –10 kJ mol^{-1} from the experimental values at 298 K. This is most likely due to the inherent deficiency of the semi-rigid docking approach of assuming the cyclodextrin as rigid. This may be overcome by developing an appropriate convenient free energy function for this type of ligand-receptor systems.

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