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A combined in silico strategy to describe the variation of some 3D molecular properties of β-cyclodextrin due to the formation of inclusion complexes

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Abstract

A powerful in silico strategy based on the combined use of two computational tools (MLP and MIFs) able to calculate and visualize 3D molecular fields can give useful information about surface properties of macromolecules involved in the mechanisms of formation of complexes. In particular, this study investigated the variation in polar/hydrophobic pattern induced on the β-CD alone (i.e. =without the ligand) by the inclusion of four ligands having different lipophilicities and small size. Results indicate that, in the presence of guests with log $P > 0$, the hydrophobicity of β-CD increases in the cavity and its surroundings on the primary face.

Keywords: β-Cyclodextrin; Inclusion complexes; In silico; Molecular fields; MLP; MIF; Lipophilicity; Hydrophobicity

1. Introduction

Modern drug design requires that ADME-Tox (Absorption, Distribution, Metabolism, Excretion and Toxicity) be addressed early in drug discovery [1]. Molecular host-guest based systems are well-known tools to optimize drug release in the body. A widely used hosts family is the cyclodextrins (CDs), which are cyclic oligosaccharides composed of 6, 7 or 8 dextrose units (α, β, and γ-CDs, respectively) joined through 1–4 bonds [2,3] (Fig. 1A). Because of their structural features (truncated-cone-shaped molecules with a hollow, tapered cavity of depth 7.9 Å, the top and bottom diameters of the cavity being 6.0 and 6.5 Å for β-CD [4], Fig. 1B), CDs tend to form inclusion complexes, and these have been used to improve solubility and bioavailability of poorly water-soluble compounds [5]. For this reason complexation with CDs is a very useful strategy for drug delivery [6,7].

An increased understanding of the forces and mechanisms involved in complexes formation of drugs with CDs should provide criteria enabling pharmaceutical scientists to design more efficacious inclusion complexes [8,9]. This study aimed to design and apply a combined in silico strategy, so as to shed light on modifications induced in some 3D molecular properties by four ligands (**2**–**5** in Fig. 1C) having different lipophilic properties and small size. It should be pointed out that such modifications are monitored on the β-CDs alone (=without the ligand).

In order to describe the properties of 3D β-CDs in qualitative and quantitative terms, two molecular fields were used [10]: the Molecular Lipophilicity Potential (MLP) [11] and the Molecular Interaction Fields (MIFs) [12–15].

The MLP spreads the molecular lipophilicity of a molecule over the Solvent-Accessible Surface (SAS) and thus its integration enables the virtual log P (i.e. the computed lipophilicity of a single conformer) to be calculated. The MIFs yield a map of the property distribution of attractive and repulsive forces between selected probes (here OH2 and DRY) and the β-CD based on energy criteria. Because of their different features, the combination of MLP with MIFs has been found to produce a more powerful computational tool to investigate 3D molecular properties than is provided by the separate application of the two methods [7,16].

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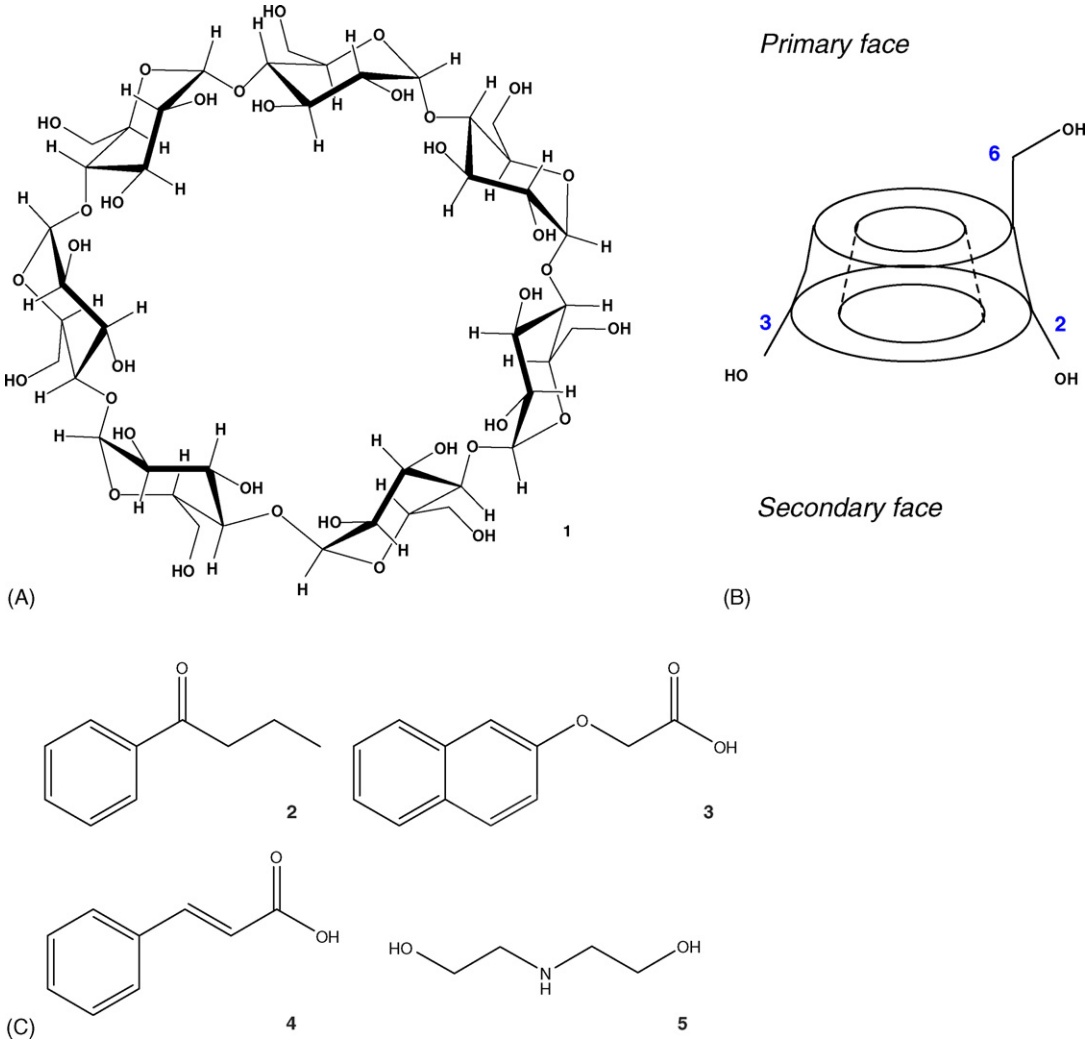

Fig. 1. (A) 2D chemical structures of β-CD; (B) diagram of 3D structure of the CD molecule. The secondary hydroxyls at positions 2- and 3- exist on the wider rim of the cone and the primary hydroxyls occupy the narrower face; (C) 2D chemical structures of ligands forming inclusion complexes with β-CDs (see text).

2. Methodology

2.1. β-CD structures retrieved from the cambridge structural database (CSD)

The following compounds were found in the Cambridge Structural Database (CSD, version 5.25; data updates April 2004): β-CD (code BCDEXD03), the complex of β-CD with butyrophenone (code DOQPOO), the complex of β-CD with naphthyloxy acetic acid (code ODEJOW), the complex of β-CD with *trans*-cinnamic acid (code XERTET) and the complex of β-CD with diethanolamine (code YIYSII). These compounds were checked with Mercury [17], saved in the Tripos mol2 format (necessary for subsequent calculations) and read in MOE [18] to carefully check the coordinates, delete co-crystallized water molecules, separate the β-CD from the ligand and add hydrogen atoms when necessary.

For β-CD alone (=obtained from BCDEXD03) two conformations were stored: **1a**, which was not minimized, and **1b**, which was minimized under MMFF94x in GB-SA conditions [9,19,20].

2.2. Conformational analysis

The conformational hypersurface of β-CDs was explored by the Hybrid Monte Carlo (HMC) module implemented in MOE [19]. The effect of solvation was taken into consideration since the solvation energy associated with the molecule's being in a continuum solvent model was included in the minimization step; in practice, a set of electrostatic corrections were calculated with the GB/SA method [20] and applied to the MMFF94x force field as implemented in MOE.

The conformer with the lowest energy resulting from the conformational analysis (**1c**) was the third conformation of β-CD (besides **1a** and **1b**, see above) stored for calculations (Table 1).

2.3. Molecular lipophilicity potential (MLP)

The Molecular Lipophilicity Potential (MLP) was calculated with Vega [21] by projecting the Broto-Moreau lipophilicity atomic constants on the solvent-accessible surface (SAS) [11]. Since MLP (see below) was designed to be applied

Table 1
Virtual log *P* values of β-CD in different conformations (see text) and of the ligands investigated, obtained by back calculation of the MLP

No.	Compound	Virtual log *P*
1a	β-CD X-ray structure (no minimization)[a]	−10.19
1b	β-CD X-ray structure (MMFF94x and GB-SA)[b]	−9.98
1c	β-CD lowest-energy[c]	−10.18
1d	β-CD extracted from the complex with butyrophenone[d]	−6.51
2	Butyrophenone	2.88
1e	β-CD extracted from the complex with naphthyloxyacetic acid[d]	−7.22
3	Naphthyloxyacetic acid	2.30
1f	β-CD extracted from the complex with *trans*-cinnamic acid[d]	−7.05
4	*Trans*-cinnamic acid	1.55
1g	β-CD extracted from the complex with diethanolamine[d]	−9.84
5	Diethanolamine	−0.66

[a] The crystallographic structure of β-CD without minimization (see Section 2 for details).
[b] The crystallographic structure of β-CD after minimization under MMFF94x and GB-SA conditions.
[c] The lowest-energy β-CD conformer resulting from conformational analysis performed by HMC simulation.
[d] Calculations were performed on β-CD alone (=without any ligand).

to the neutral species, ionizable ligands (**3** and **4**) were analyzed in their undissociated state. This approximation is acceptable because the negative charge of **3** and **4** is localized almost entirely on one side of the molecules.

2.4. Molecular interaction fields (MIFs)

A MIF is a collection of energy values calculated from the sum of the attractive and repulsive forces between a molecule (the target) and an interacting partner (the probe), positioned in a lattice of points (or nodes) surrounding the target. Nodes with negative energy values correspond to favorable interactions between the molecule and the probe, and vice-versa; using different probes the predisposition of the target toward various interaction types can be evidenced.

Two probes were chosen for this study, water (OH2) and the hydrophobic probe (DRY), and the corresponding MIFs were calculated using the program GRID [22] and default parameters, except for the number of planes of grid points per Angstrom (NPLA directive) which was set to two for both probes.

The MIFs were then exported using readable format and submitted to an in-house program to obtain some numerical descriptors (the Volsurf software [10] automatically extracts numerical information present in MIFs but it is not specifically designed for the molecular visualization of these data, see below).

Briefly, our program counts the number of points (known as final points) with energy equal to or below a selected energy cutoff value (always negative). These points are located around the target molecule and determine regions of favorable interactions with the probe; the choice of appropriate cutoffs enables different information to be obtained, see Section 3. The number of points is proportional to the volume of the interaction region and can thus be used as a numerical descriptor. For improved visualization of the results in MOE (and for applications other than that reported here) final points that are close together in the original grid are joined together to form cubes; a cluster is assumed to form when at the least two cubes have a common side. Lastly, the software lists the number of final points, cubes and clusters in a text file and the information necessary for their visualization is stored in a .pdb file easily readable by any molecular modelling package. The in-house program was written in PERL and can run on either Linux or Windows (equipped with the CYGWIN environment [23]) based machines.

All calculations were performed on a Linux based dualprocessor Appro1124 server and on standard PCs operating with Microsoft Windows XP.

3. Results

3.1. β-CD

The first step of this study consisted in determining relevant conformations for β-CD. In particular, three conformations were considered: (a) the crystallographic structure without minimization (**1a**), (b) the crystallographic structure after minimization under MMFF94x and GB-SA conditions (to take the solvent into account) (**1b**) and (c) the conformer of lowest energy resulting from a conformational analysis (**1c**) performed by an HMC simulation (the method used is similar to one of those cited by Lipkowitz [24]). This preliminary operation is required since the active conformation at any receptor (for example β-CD) is unknown, and thus more than one possibility must be explored. In particular, X-ray data might be affected by packaging artifacts and the minimum energy structure (defined as the structure with the 3D geometry placing the molecule at the lowest point on the potential energy hypersurface) depends on a number of factors, such as starting conformations features and calculation tool adopted.

Similar values of virtual log *P* (about −10) were obtained for **1a**–**1c** (Table 1). In addition the limited variability of the lipophilicity was confirmed by virtual log *P* data calculated for the 51 conformers obtained from the HMC simulation (data not shown). It must be pointed out that the MLP by Gaillard et al. [11] has not been validated for very low log *P*s (<−3) and thus its application to β-CDs may be of doubtful validity. However, this study only analyzed a small number of molecular geometries of the same compounds (β-CD) and thus the difference in log *P* among conformations was of more interest than its absolute value.

At visual analysis, the lipophilicity pattern given by MLP (Fig. 2A) is fairly similar to the results described by Lichtenthaler and Immel [7], since the 2-OH/3-OH side of the macrocycles is distinctively hydrophilic (blue dots), the opposite narrower opening made up of the 6-CH_2OH groups shows few hydrophobic points (yellow dots) and the cavity surface is partially hydrophobic rather than being fully hydrophobic as has often been reported [4,8].

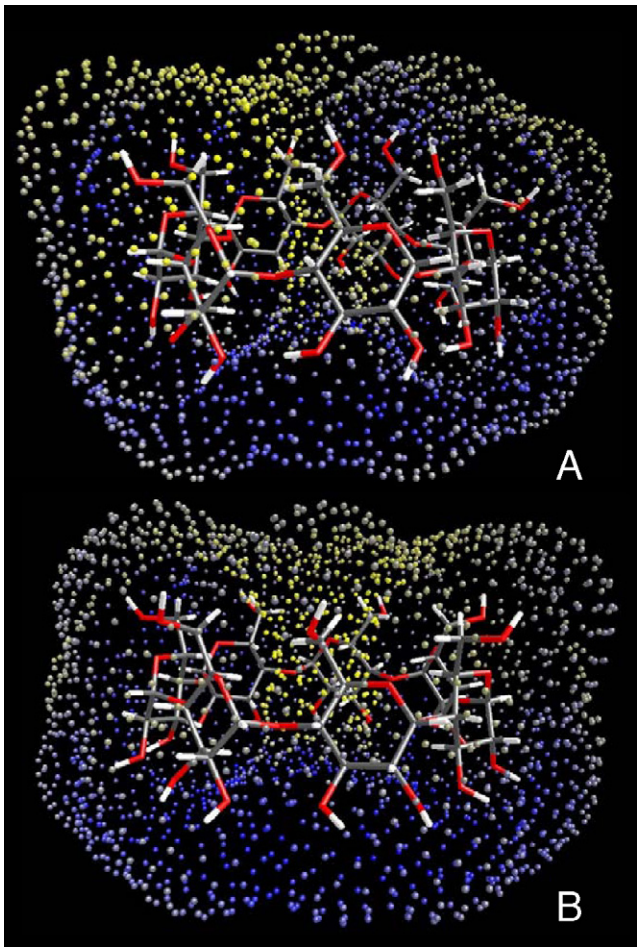

Fig. 2. MLP representation. Color coding follows a scale from the most polar regions (blue) to the most hydrophobic region (yellow). Side view with the 2-OH/3-OH side pointing downwards (larger opening of the torus) and the 6-CH_2OH upwards (smaller opening of the torus). (A) **1a**; (B) **1d**.

A validation run was performed to check the efficacy of MIFs in investigating CDs; this determined whether the presence of water molecules in their observed crystallographic position in the β-CDs would be detected as favorable in energy terms, when GRID was applied using the OH2 probe [25]. The results are given in Fig. 3, which shows a good superposition in the cavity between crystallized water molecules (red balls) and GRID minima (blue cubes, for cubes definition see Section 2).

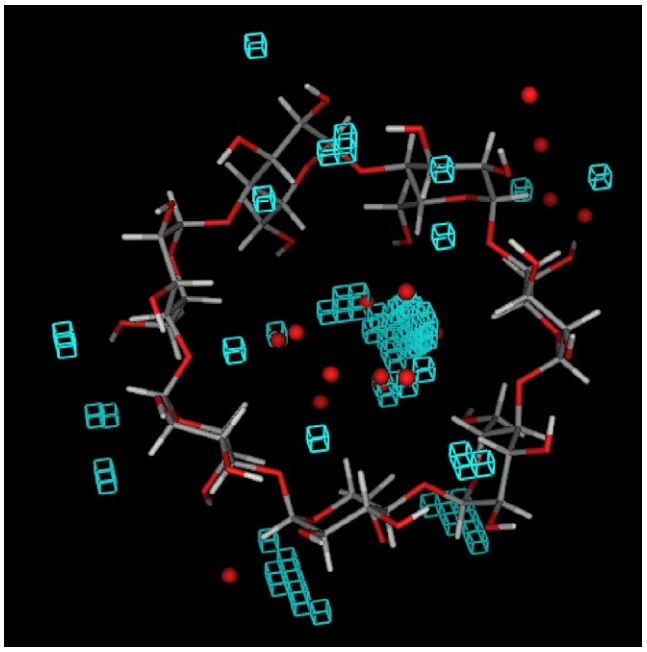

Fig. 3. GRID validation for **1a**: red balls represents crystallized water molecules, blue cubes indicate the GRID hydrophilic regions (=obtained with the water probe) at −5.0 kcal/mol. The structure is shown perpendicular to the mean ring plane of the macrocycle and is viewed through the large opening of the conically-shaped molecule.

To obtain results comparable with MLP data, two MIFs were used: the hydrophilic field generated by the OH2 probe and the hydrophobic field generated by the DRY probe. Hydrophilic regions are defined as the molecular envelope which is accessible to and attracts water molecules. In general, hydrophilic descriptors computed from molecular fields of −0.2 to −1.0 kcal/mol account for polarizability and dispersion forces, whereas descriptors computed from molecular fields of −1.0 to −6.0 kcal/mol account for polar and H-bond donor–acceptor regions. Hydrophobic regions may be defined as the molecular envelope generating attractive hydrophobic interactions. The usual energy range of hydrophobic interactions is from 0.0 to −2.0 kcal/mol [10].

Since the volume of MIFs varies with the energy cutoff value (see Section 2), −5.0 kcal/mol was taken as a cutoff for the hydrophilic field and −0.05 kcal/mol for the hydrophobic field, and a specific in-house program (see Section 2) was used to

Table 2
MIFs numerical results for β-CD in its different conformations (see text). Calculation were performed on β-CD alone (=without any ligand)

No.	OH2 (−5 kcal/mol)			DRY (−0.05 kcal/mol)			r (DRY/OH_2)[a]
	Final points	Cubes	Clusters	Final points	Cubes	Clusters	
1a	1863	74	28	59	0	0	0.03
1b	1984	106	31	120	1	1	0.06
1c	1893	120	24	78	0	0	0.04
1d	1969	64	42	404	54	14	0.21
1e	1900	62	40	380	54	8	0.20
1f	1844	58	34	356	45	7	0.19
1g	1731	52	28	141	14	2	0.08

[a] Ratio between number of dry points and number of water points.

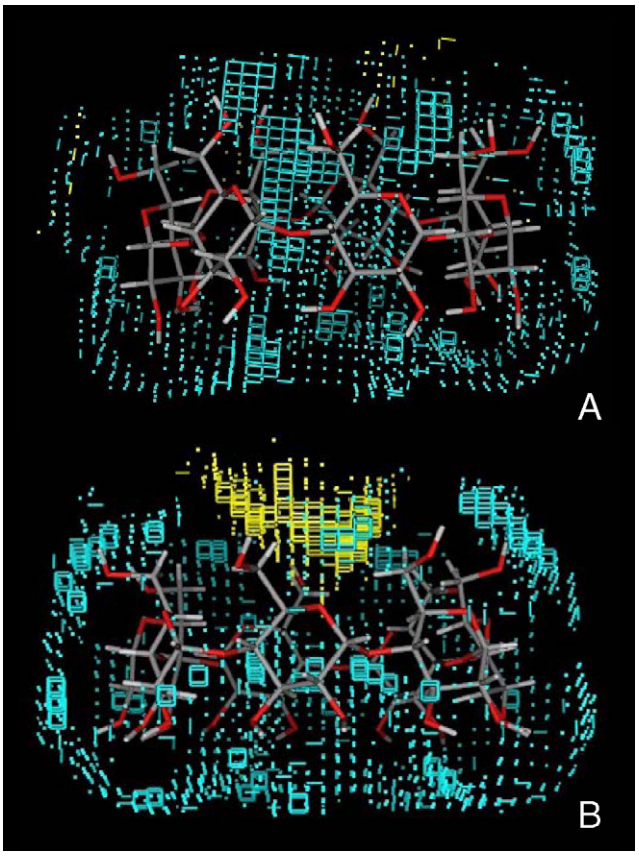

Fig. 4. MIFs visualization: hydrophilic regions (blue) at −5.0 kcal/mol and hydrophobic regions (yellow) at −0.05 kcal/mol. The orientation is such that the 2-OH/3-OH side points downwards (larger opening of the torus) and the 6-CH_2OH points upwards (smaller opening of the torus): (A) **1a**; (B) **1d**.

compute the hydrophobic and hydrophilic contributions at the chosen energy levels. MIFs analysis was both numerical and graphic (Table 2, Fig. 4A) and showed that hydrophobic (yellow) regions were very small and located around the primary face (Fig. 1B), blue regions were widely dispersed around the secondary face and also inside the cavity [8].

The balance between the hydrophobic and the hydrophilic regions was evaluated from the ratio between the number of final dry points and the number of final water points (r (DRY/OH2)). The higher this ratio, the larger the extension of the hydrophobic regions. Again no significant difference was found between **1a**, **1b** and **1c**, since their r (DRY/OH2) values were very similar (Table 2).

3.2. β-CD in inclusion complexes

The complexes of β-CD with butyrophenone (**2**), naphthyloxyacetic acid (**3**), *trans*-cinnamic acid (**4**) and diethanolamine (**5**) were selected from the CSD database because of the different lipophilicities of the ligands. Virtual log *P* indicates that ligands **2**–**5** cover a lipophilicity range of about 3.5 logarithm units, as shown in Table 1 (CLOGP and other softwares also gave the same results, data not shown). In particular compounds **2**, **3** and **4** are lipophilic (log $P > 0$) and **5** is hydrophilic (log $P < 0$).

On the basis of previous results (see above), the crystallographic structures of the β-CDs in selected inclusion complexes were used without further minimization. The following codes were adopted: β-CD extracted from the complex with butyrophenone = **1d**, with naphthyloxyacetic acid = **1e**, with *trans*-cinnamic acid = **1f** and with diethanolamine = **1g**.

Log *P* values for **1a**–**1c** were lower than those for **1d**–**1g** (Table 1) and the more lipophilic the ligand, the higher the log *P* of the corresponding β-CD. In particular visual inspection of the lipophilicity potential showed the cavity of **1d** (Fig. 2B) to be more lipophilic than that of **1a** (Fig. 2A). The same was true for **1e** and **1f** (to a lesser extent), but not for **1g**, which had about the same MLP profile as did **1a**–**1c** (not shown).

MIFs data for **1d**–**1g** are given in Table 2. In agreement with MLP data (Table 1), the extension of the hydrophobic regions (as expressed by r (DRY/OH_2)) was larger for CDs extracted from the complexes with lipophilic ligands than for **1a**–**1c**, but for **1g** (the corresponding ligand, **5**, had a negative log *P* value) r (DRY/OH2) was similar to those of **1a**–**1c**.

Graphic analysis of MIFs gave similar information as did MLP (Fig. 4B reports molecular fields for **1d**): the zone of the primary face around the cavity was hydrophobic in the presence of lipophilic ligands. Conversely, as Fig. 5 shows, **3** and **4** were positioned such that their polar moieties pointed towards the most hydrophobic region (results for MLP were similar). For **2**, **3** and **4**, the aromatic portion was outside the CD cavity.

At analysis in greater depth, as shown in Fig. 6A and B for naphthyloxyacetic acid, the aromatic moiety was located in a region not fully hydrophilic, since if the energy level of the hydrophobic MIF was increased (thus including less relevant interactions), a yellow region was also present around the aromatic ring (Fig. 6B).

4. Discussion

Lichtenthaler and Immel [7] have shown that the mean molecular geometry parameters of solid-state CD structures and their inclusion compounds are within normal ranges. In this study we demonstrate that, despite limited geometrical modifications, the formation of a complex considerably changes 3D molecular properties of β-CDs as demonstrated by the increased lipophilic content around the primary face and the cavity.

From these results, ligands might be expected to be positioned to satisfy the reciprocal interplay between polar and hydrophobic patches on the host and guests molecules, as has been proposed [16], but this did not occur for the ligands investigated in the present study.

There are two main possible reasons for this behaviour, which may act separately or in combination. First, a steric hindrance could limit the accessibility of the β-CDs primary (more hydrophobic) face to the ligands' aromatic portions. The second explanation may be clarified by re-analyzing the mechanism described for the complex formation [2], which may be compared with a typical hydrophobic interaction process [4]. In particular, in an aqueous solution, the apolar

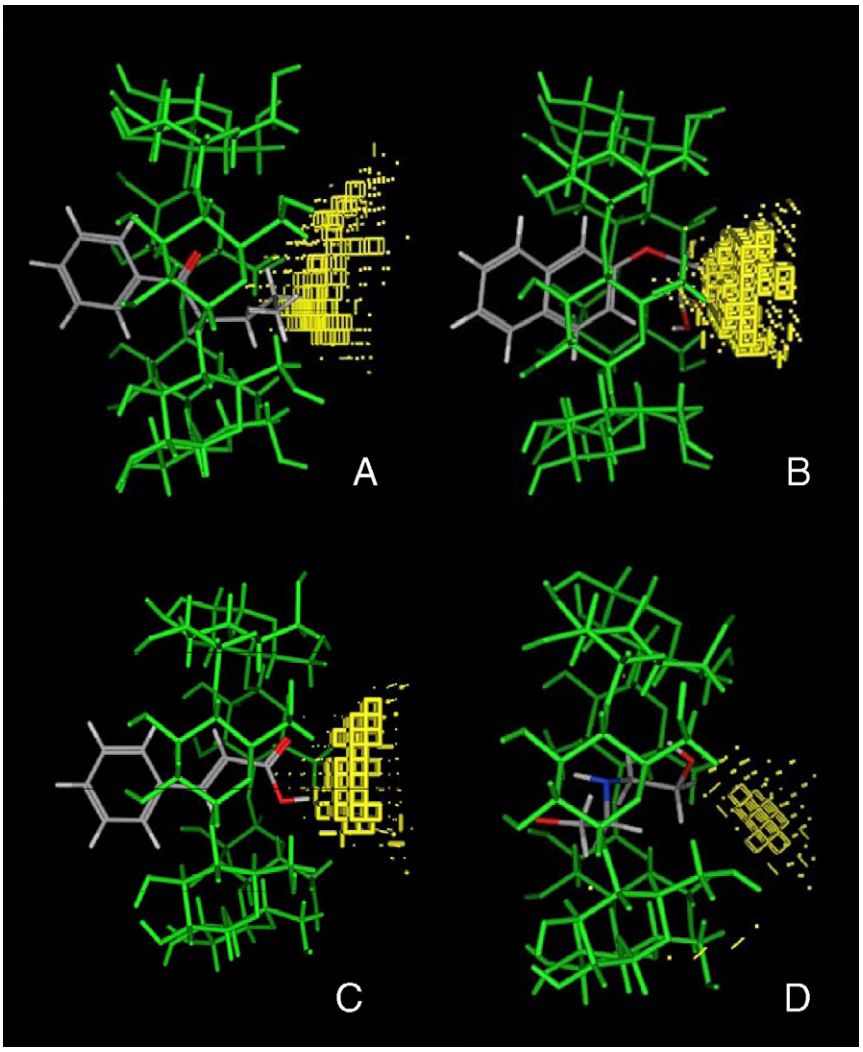

Fig. 5. Inclusion complexes of ligands **2**–**5** (standard colors) with β-CD (in green). Dry regions are in yellow (−0.05 kcal/mol): (A) butyrophenone (**2**); (B) naphthyloxyacetic acid (**3**); (C) *trans*-cinnamic acid (**4**); (D) diethanolamine (**5**).

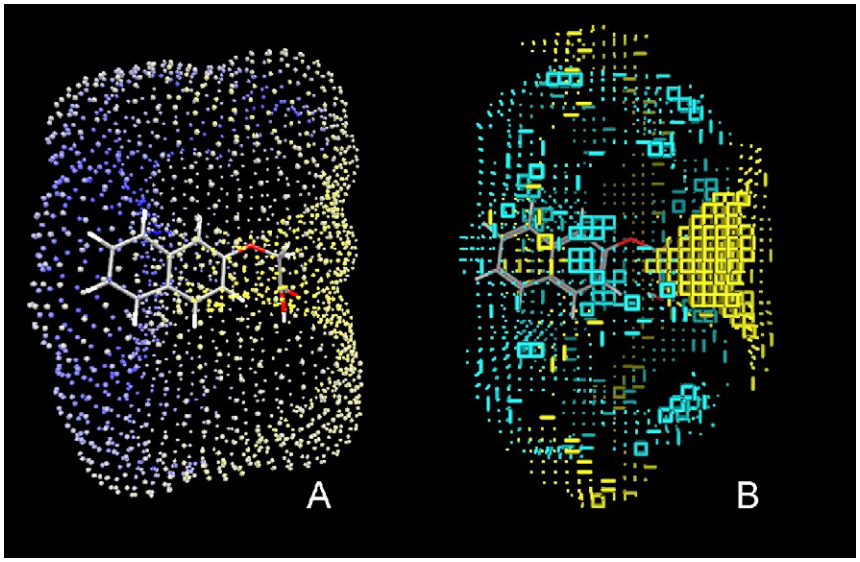

Fig. 6. Inclusion complex of β-CD with **3**. 3D molecular fields results are shown for **1e**: (A) MLP. The color coding follows a scale starting from the most polar regions (blue) to the most hydrophobic regions (yellow); (B) MIF: hydrophilic regions (blue) at −5 kcal/mol and hydrophobic regions (yellow) at −0.005 kcal/mol.

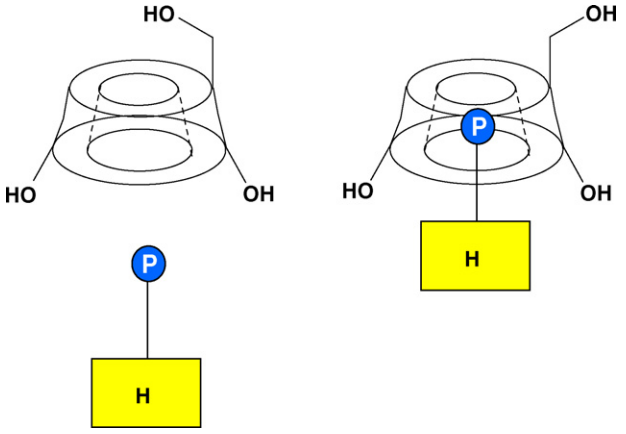

Fig. 7. Diagram of hypothesized entrance of a generic compound into the β-CD cavity, as deduced from the results reported here. In particular the presence of a polar group (*P*) in the ligand structure might facilitate entry. Entrance is facilitated if it is from the secondary face, which is the largest. Once the ligand has entered, the cavity space might be increased by opening the primary oxygens, thus extending the hydrophobic region and facilitating accommodation of the hydrophobic (H) portion of the ligand.

cyclodextrin cavity is assumed to be occupied by water molecules, which are unfavorable from the energy standpoint (polar–apolar interaction) and therefore can be readily substituted by appropriate less polar guest molecules. Indeed, the CD cavity is mainly hydrophilic and thus the presence of a polar group in the ligand structure could help entry into the cavity. Entrance is facilitated if it occurs from the secondary face, which is the largest face. Once the ligand has entered, the CD cavity space would be increased if the oxygen opened, extending the hydrophobic region. This would enable a hydrophobic ligand to be accommodated more easily.

Fig. 7 shows a schematic representation of this hypothesis which is also in agreement with the experimental results recently published by Uccello-Barretta et al. [9] who found that molsidomine preferentially binds β-CD by the morpholine moiety (more polar) rather than the ethylic group (more hydrophobic).

We also sought stability constants (log *K*) for 1:1 inclusion complexes of pairs of compounds that differ only in the presence of a polar substituent (i.e. benzene and phenol). If our assumption is correct, these experimental data should show that the presence of a polar moiety allows the derivative (i.e. phenol) to have a higher log *K* than the parent (i.e. benzene). Among the data listed by Rekharsky and Inoue in their comprehensive review [4], we found log *K* data for two pairs of compounds: benzene (about 2.1) and phenol (about 3.4) and naphthalene (about 2.8) and 1-naphtol (about 3.1). The hypothesis we put forward here was verified in each case. Unfortunately, since the paucity of reliable data (the quality of experimental log *K* is often not comparable when obtained in different laboratories) we cannot extend our demonstration to more pairs of compounds.

Lastly, these results are of additional significance because they are the result of the combination of two different computational tools, each having its own weakness and strengths. In particular, the MLP approach is much simpler than the GRID based tool and can furnish good preliminary indications about the topic under investigation, but for deeper insight into the problem the use of GRID is mandatory, chiefly because it can be tailored to specific requirements.

5. Conclusions

This study demonstrates the need for new computational methodologies in molecular modelling based on the combination of two or more existing methods. The combined use of two in silico strategies cannot of course replace experimental proofs, but it does provide a more powerful tool than the separate use of any one software package.

The efficacy of this approach is demonstrated here by applying it to the study of interactions of β-CDs with a series of four ligands. Work is in progress to extend the study to larger ligands for which no crystallographic data have yet been published, in order to extract information on their log *K* values. The final step of the study will be to apply the new strategy to derivatized non-ionic and ionic β-CDs.

Acknowledgement

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