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ORIGINAL ARTICLE

Binding, and thermodynamics of β-cyclodextrin inclusion complexes with some coumarin laser dyes and coumarin-based enzyme substrates: a simulation study

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Abstract
This paper addresses modelling the nature of interactions between β-CD and some coumarins including recently reported novel sulphur analogues to form inclusion complexes of appealing medicinal, photochemical and photophysical properties. The binding energy and the total stabilization energy (EONIOM) are used to confirm the most favorable inclusion complex structure. Thermodynamic parameters reveal exothermic inclusion reaction in gas phase. Thermal stability of fluorescent enzyme substrate of coumarin nucleus increases in the order: gas < cyclohexane < water, indicating better stability in water. Furthermore, molecular characteristics such as optimized geometries, MO’s and electrostatic potential energy map surfaces and energies are reported and correlated with some reactivity indices. Our results validated the experimentally available data reported in the literature. Inclusion complexes of β-CD with coumarins should result in improving its laser efficiency in environmentally benign aqueous medium.

Keywords Coumarins · β-Cyclodextrin · DFT · ONIOM

Introduction
Photophysical properties of 7-hydroxy-4-methylcoumarin [4-methylumbelliferone; (4-MU)] and some coumarin derivatives has been investigated extensively [1–25]. Recently, it has been highlighted that tautomers of 4-MU due to water bridging proton transfer from the hydroxyl group to the carbonyl oxygen acceptor can occur [18–22]. Light absorption results in the population of a locally excited (LE) first singlet state (S1, ππ*) mixed with nπ*. This is characterized by extensive non-radiative energy dissipation on the sub-nanosecond time scale, which shows a sensitivity to environmental factors (solvent polarity and hydrogen-bonding ability) that may be exploited profitably in various systems of industrial and biological importance. Moreover, excellent linear correlations were established between some photophysical properties of 4-MU and some solvent properties. The results were discussed on the basis of elementary quantum chemical data obtained by application of the semiempirical INDO, CNDO/S-CI, ZINDO-CI/S methods [1, 23, 25]. Generally, more reliable quantum mechanical computations of vertical and adiabatic excitations of neutral, deprotonated and protonated form of HCs have been reported in the gas and liquid phases [26–30]. It is well known that these dye lasers are suitable sources of UV and visible light. However, the use of dyes can generate some problems since dyes undergo undesirable processes such as aggregation and photo-oxidation, which could lower the efficiency of emission. Furthermore, the majority of laser dyes are poorly soluble in the environmentally benign water solvent. It is recommended that some of the above mentioned problems could be overcome by applying cyclodextrins.

Recent published work reports on the photophysical properties of 7-mercapto-4-methylcoumarin in protic and nonprotic solvents [31]. Despite their potential applications as optical whitening agents, only few publications reported the
fluorescent 7-mercaptocoumarins [32, 33] and no data are available on the nature of their inclusion complexes.

In this work, we aimed to simulate properties of seven coumarins; namely 4-MU and some of its sulphur analogues together with their tautomers as well as two related enzyme substrate to help elucidate the stabilities of their inclusion complexes with β-cyclodextrin (β-CD). The main focus is on the inclusion complexes of β-CD and the seven coumarins depicted in Fig. 1. The ways of interactions and the thermodynamic stability of the complexes will be simulated using the semi-empirical PM3, PM6, methods and ONIOM (Own N-layer Integrated molecular Orbital molecular Mechanics) combinations (APFD/6-311G(d,p): PM3 and APFD/6-311G(d,p): PM6).

The research results reported will provide further theoretical information for possible application of selected coumarins in medicine and food industry as well as in dye laser and fluorescence sensing.

Computational details

The calculations considered the molecules shown in Fig. 1. Spartan’16 parallel software was used [34] for optimization of the ground-state geometries of individual free coumarins and cyclodextrin (starting the optimization from the available X-ray structure of β-CD), using the DFT method with the functional EDF2 and basis set 6-311G(d,p). The motivation of using EDF2 functional (Empirical Density Functional 2) was to obtain results that are general and less costly [35]. Running calculations with the EDF2/6-311G(d,p) model is computationally efficient method and reliable enough to describe the properties of coumarins with sufficient accuracy. The results were verified with experimental findings available in the literature. Based on the already published orientations, the initial configurations of these inclusion complexes were constructed manually. Then, inclusion complexes were optimized using the semi-empirical PM3 [36], PM6 [37], and ONIOM [38, 39] (APFD/6-311g(d,p): PM3 and APFD/6-311g(d,p): PM6) methods. Gaussian 16 version A.03 [40] was used. APFD hybrid DFT method includes dispersion [41] was used for higher layer in ONIOM job. Semi-empirical PM3 or PM6 methods were used for outer layer (β-CD). Solvent effects were treated using CPM model [42].

Results and discussion

Molecular properties and reactivities

Simulated molecular properties of the studied compounds are listed in Table 1. Reactivity indices are calculated from the HOMO–LUMO energies [43–48].

The data listed in Table 1 reveals the following: (a) chemical potential (µ) increases in the order 4 < 2 < 5 < 6 < 1 < 7 < 3, and (b) EHOMO and nucleophilicity N increases in the order 7 < 3 < 1 < 6 < 4 < 5 < 2. Relative to the tautomer 4-MU (1), tautomer (2) is less stable. The tendency of (1) to resist electron release as measured by (µ) is larger than compounds (2), (4), (5) and (6), while (7) and (3) have the highest chemical potential. The most nucleophilic is the keto form of 4-MU (2) and the least is the enzyme substrate (7), which quantitatively supports the qualitative conclusion based on the molecular structure of these compounds.

Furthermore, ESP maps [49–52] are presented in Fig. 3. ESP map represents the total electron distribution mapped with the electrostatic potential energy over the whole...
molecule. The binding sites of a molecule could be visualized by ESP map, which is important to identify the interaction locations within a molecule. Gradual potential change is represented by color codes: the bluest is the most positive region, in kJ/mol, while the reddest region is that with most negative potential.

Figure 2 shows that glucanase enzyme substrate (7) exhibits most relaxed (smallest positive ΔESP value) molecular structure, which is characterized by smallest nucleophilic index, hardest electron donation character (η) and almost largest HOMO–LUMO mean value (μ).

The enol (1) or thiol (3) tautomers are more energetically stable than the corresponding tautomers (2) or (4), respectively. This is due to thermodynamic stabilization provided by aromaticity, see Table 1.

### Inclusion complexes

β-Cyclodextrin and its functionalized derivatives are very interesting compounds capable to include inside its cavities a tremendous number of valuable compounds via hydrogen bond formation and dispersion forces. Thus, inclusion complexes of cyclodextrins are still attracting the intention of researchers from different fields. Profitable exploitation of such compounds in pharmaceutical and food industry, in analytical and organic chemistry are remarkable. Computational chemical studies of inclusion complexes could support the experimental findings and provide highly valuable information regarding the structure, spectroscopic characteristics and thermodynamic stabilities [38, 53, 54]. The research results presented and discussed below will provide a deeper theoretical insight for the potential application of coumarin derivatives under investigation in photochemical, medicinal and food industries. Therefore, this study is of theoretical and practical significance in deepen the investigation.

### β-CD with simple hydroxyl and mercapto derivatives

In this section, we report on the theoretical studies on 1:1 CD inclusion complexes of seven coumarin derivatives depicted in Fig. 1. It is previously found that 4-MU:CD of 1:1 stoichiometry is of considerable thermodynamic stability [53, 54]. However, through an analysis of the pH-dependent UV absorption and fluorescence spectra of 7-hydroxycoumarine derivatives, it was experimentally found that the proton dissociation abilities of these guests undergo negligible effects of the β-CD inclusion in both the ground-state and the excited singlet-state due to exposure of coumarins to the aqueous phase [54]. Our obtained thermodynamic parameters (Go, and Ho), computed from semiempirical computations, validated that the inclusion process of (1) into CD was a spontaneous and exothermic process validating previously reported data [53, 54]. Here we significantly extend the studies to include sulphur derivatives as well as two enzyme substrates (6) and (7) using higher level computational model. Thermodynamic parameters, computed from oniom and semiempirical computations, validated that the inclusion process of each of the seven derivatives into CD is a spontaneous exothermic process. The structure information obtained theoretically showed that the each coumarin molecule, regardless of its size, could reside inside the molecular cavity of the CD, which has asymmetry axis parallel to the transition moment axis of the guest molecule.

Interactions between β-CD and the coumarins 1–5 forming inclusion complexes are simulated using the semiempirical PM3, PM6, and ONIOM (APFD/6-311G(d,p); PM3 and APFD/6-311G(d,p); PM6) methods. We will report here only oniom with the higher level pm6 results noticing that same trends in energy variation are obtained in case of applying PM3. APFD hybrid DFT method includes dispersion is used for higher layer in ONIOM job while semiempirical PM3 or PM6 methods are used for outer layer (β-CD). Geometry optimized inclusion complexes are represented in Figs. 3 and 4.

The binding energy and the total stabilization energy (E^{ONIOM}) were used to confirm the most favorable inclusion complex structure. Thermodynamic parameters values show that the inclusion reaction is exothermic process in gas phase as well as in non-polar cyclohexane and in water, detailed discussion will be given later. Moreover, as will be seen below.
the analysis demonstrates that the hydrogen bonds interactions are of type C–H⋯O.

The well-known stabilization energy $\Delta E_{\text{stab}}$ (sometimes called binding energy, $E_{\text{bind}}$) is defined as:

$$\Delta E_{\text{stab}} = E_{\text{Complex}} - (E_{\text{CD}} + E_{\text{Cou}})$$

where $E_{\text{Complex}}$, $E_{\text{CD}}$, $E_{\text{Cou}}$ are the total energy of the geometry optimized complex, free $\beta$-CD and the guest coumarin molecules, respectively the data are collected in Table 2. The stabilization Gibbs free energy change ($\Delta G_{\text{stab}}$) is defined similarly:

$$\Delta G_{\text{stab}} = G_{\text{Complex}} - (G_{\text{CD}} + G_{\text{Cou}})$$

Both $\Delta E_{\text{stab}}$ and $\Delta G_{\text{stab}}$ do not include basis set superposition error (BSSE) corrections because BSSE is not available in ONIOM calculations. Applying PM6 method we get the following numerical data (Table 3) for the Gibbs free energy change for molecules (1–5).

Negative values of $\Delta E_{\text{stab}}$ or $\Delta G_{\text{stab}}$ reveal thermodynamically stable inclusion complex.

The data compiled in Tables 2 and 3 point to stable inclusion complexes. Keto tautomer of 4-MU (2) is less stable than the enol form (1). And the same applies for molecule (3) SH and its (4) tautomer.

Further support could be obtained from more accurate computations by using multilayer technique included in...
the Gaussian package. In the two-layered ONIOM method [55–57], the complex is divided into an inner and an outer layer. The inner layer consists of the coumarin derivative calculated at a higher level of theory, and the \( \beta \)-CD comprises the outer layer calculated at a lower level of theory, which yield a consistent energy expression with similar accuracy to a higher-level calculation in the full system [58]. The full system is called “real” treated with a lower level of theory; the inner layer is termed “model” treated with both the lower and the higher levels of theory. The total stabilization energy (\( \text{E}^{\text{ONIOM}} \)) is expressed as:

\[
\text{E}^{\text{ONIOM}} = \text{E}^{\text{high, model}} + \text{E}^{\text{low, real}} - \text{E}^{\text{low, Model}}
\]

where \( \text{E}^{\text{high, model}} \) is the energy of the inner layer guest molecule at a higher level [here DFT model, \( \text{E}^{\text{low, real}} \) is the energy of the full system (inclusion complex)] at the lower level (here at PM3 or PM6), and \( \text{E}^{\text{low, model}} \) is the energy of the outer layer \( \beta \)-CD at the lower level. The computed data obtained for the most stable geometries are summarized in Table 4. It is interesting to note that two distinct stable geometries are noticed for the enzyme substrate (7) the first one with coumarin moiety (head) located inside CD cavity and the second with the cellobioside (tail) moiety inside the cavity. The latter is more stable by about 20 kJ/mol.

Thermodynamic results obtained from oniom computations favor the formation of the inclusion complex of the enol form, supporting the conclusions that could be drawn from the inspection of the thermodynamic data obtained from the pure semiempirical methods given in Table 4.

It is noteworthy mentioning that at higher computation model, more accurate thermodynamic results are obtained. In case of oniom computations, the increasing number of H bonds formed between coumarin and the CD molecule could be used to rationalize the obtained stability. Generally, increasing number of H-bond formation between the guest and the host moieties should obviously contribute to the stability. O–H⋯O or C–H⋯O Hydrogen bonding is assumed in cases where the O⋯H bond distance is shorter than 2.45 Å. Some of the H-bond distance is shorter than 1.8 Å, efficiently limiting the flexibility of the whole guest molecule. The main contribution into the non-specific interactions between coumarins and CD will have the electrostatic interactions as well as a minor contribution from Van der Waals forces could also affect the stability of the inclusion complexes. Figure 4 shows H-bonding in the inclusion complexes of some molecules as examples. The interplay between H-bonding forces and
other weak forces could rationalize the obtained trend in the thermodynamic stability of the coumarins under investigation. Comparing the structure-stability, 4-MU (1) is the most stable followed by SMe (5) derivative then SH (3) derivative. This is consistent with the stability of enol (1) or thiol (3) relative to the corresponding tautomers (2) or (4), respectively, see Table 1.

**β-CD and Enzyme Substrates**

There are no experimental data available for such coumarin-based enzymes-CD complexes. The performed computations may stimulate interest in carry out experimental investigations.

Interactions between β-CD and the enzyme substrate (6) and (7) forming inclusion complexes (Figs. 3, 4) were
simulated using less expensive (but traditionally used) methods; semi-empirical PM3, and ONIOM (B3LYP/3-21G: PM3). Table 5 contains the data obtained for molecule (6) complex with \( \beta\text{-CD} \).

Ongoing from gas phase through non-polar cyclohexane to water the enzyme substrate (6) becomes more thermally stable. The gain due to cyclohexane and water solvation is given in Table 5 and increases as the medium becomes more polar. This is due to solvent-induced change in the electronic character of this polar molecule [53]. Unfortunately, no experimental studies are reported about the inclusion complexes of the enzyme substrates studied. However, we provided the theoretical characteristics, which are valuable for experimental design.

**Conclusions**

Inclusion complexes between \( \beta\text{-CD} \) and 4-MU and some of its related sulphur as well as enzyme substrate were simulated using the semi-empirical PM3, PM6, methods and ONIOM combinations (APFD/6-31G(d,p): PM3 and APFD/6-31G(d,p): PM6), as well as oniom(B3LYP/3-21G:PM3). The binding energy and the total stabilization energy \( (E_{\text{ONIOM}}) \) were used to confirm the most favorable inclusion complex structure. Thermodynamic parameters values show that the inclusion reaction is exothermic in gas phase and in solutions. Effect of solvent polarity on the thermal stability of enzyme substrate is of higher energy-driven in water than in non-polar solvent or in the gas phase. Our theoretically obtained data validated the reported experimental findings. Additionally, optimized geometries, reactivity indices and electrostatic potential energy maps (ESP) map surfaces and energies are reported and correlated with structure change. To our knowledge, this is the first comprehensive simulation study on such coumarin derivatives.

**Table 2** Thermal parameters in kJ/mol of optimized inclusion complexes of \( \beta\text{-CD} \) with enol (1) - keto (2) tautomers of 4-MU and sulphur derivatives computed at PM6 semiempirical level (energy of free \( \beta\text{-CD} = -6547.83 \))

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Optimized free molecules/tautomer</th>
<th>Optimized inclusion complex</th>
<th>( \Delta E_{\text{stab}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-408.23</td>
<td>-7031.53</td>
<td>-75.47</td>
</tr>
<tr>
<td>2</td>
<td>-298.27</td>
<td>-6910.28</td>
<td>-64.18</td>
</tr>
<tr>
<td>3</td>
<td>-213.83</td>
<td>-6824.09</td>
<td>-62.43</td>
</tr>
<tr>
<td>4</td>
<td>-65.68</td>
<td>-6657.53</td>
<td>-44.02</td>
</tr>
<tr>
<td>5</td>
<td>-222.01</td>
<td>-6829.39</td>
<td>-59.55</td>
</tr>
</tbody>
</table>

\( \Delta E_{\text{stab}} \) decreases: 1 > 2 > 3 > 5 > 4

**Table 3** Calculated (PM6 semiempirical) stabilization Gibbs free energy change \( \Delta G_{\text{stab}} \) defined by: \( \Delta G_{\text{stab}} = G_{\text{Complex}} - (G_{\text{CD}} + G_{\text{Cou}}) \), where \( G_{\text{CD}} = -1.085516 \text{ au} \)

<table>
<thead>
<tr>
<th>Molecule</th>
<th>( G_{\text{Complex}} \text{ (au)} )</th>
<th>( G_{\text{Cou}} \text{ (au)} )</th>
<th>( \Delta G_{\text{stab}} \text{ (kJ/mol)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1.443054</td>
<td>-0.003286</td>
<td>-930.00</td>
</tr>
<tr>
<td>2</td>
<td>-1.546401</td>
<td>-0.044068</td>
<td>-1094.68</td>
</tr>
<tr>
<td>3</td>
<td>-1.455559</td>
<td>-0.025026</td>
<td>-905.84</td>
</tr>
<tr>
<td>4</td>
<td>-1.513448</td>
<td>-0.081058</td>
<td>-910.72</td>
</tr>
<tr>
<td>5</td>
<td>-1.434180</td>
<td>-0.044089</td>
<td>-799.66</td>
</tr>
</tbody>
</table>

\( \Delta G_{\text{stab}} \) decreases in the order 2 > 1 > 4 > 3 > 5

simulated using less expensive (but traditionally used) methods; semi-empirical PM3, and ONIOM (B3LYP/3-21G: PM3). Table 5 contains the data obtained for molecule (6) complex with \( \beta\text{-CD} \).

Ongoing from gas phase through non-polar cyclohexane to water the enzyme substrate (6) becomes more thermally stable. The gain due to cyclohexane and water solvation is given in Table 5 and increases as the medium becomes more polar. This is due to solvent-induced change in the electronic character of this polar molecule [53]. Unfortunately, no experimental studies are reported about the inclusion complexes of the enzyme substrates studied. However, we provided the theoretical characteristics, which are valuable for experimental design.

**Conclusions**

Inclusion complexes between \( \beta\text{-CD} \) and 4-MU and some of its related sulphur as well as enzyme substrate were simulated using the semi-empirical PM3, PM6, methods and ONIOM combinations (APFD/6-31G(d,p): PM3 and APFD/6-31G(d,p): PM6), as well as oniom(B3LYP/3-21G:PM3). The binding energy and the total stabilization energy \( (E_{\text{ONIOM}}) \) were used to confirm the most favorable inclusion complex structure. Thermodynamic parameters values show that the inclusion reaction is exothermic in gas phase and in solutions. Effect of solvent polarity on the thermal stability of enzyme substrate is of higher energy-driven in water than in non-polar solvent or in the gas phase. Our theoretically obtained data validated the reported experimental findings. Additionally, optimized geometries, reactivity indices and electrostatic potential energy maps (ESP) map surfaces and energies are reported and correlated with structure change. To our knowledge, this is the first comprehensive simulation study on such coumarin derivatives.

**Table 4** Thermodynamic data for the inclusion complexes of the molecules studied with \( \beta\text{-CD} \) using different methods: # oniom (APFD/6-31G(d,p): PM6) for molecules (1–5) and# oniom (B3LYP/3-21G:PM3) for molecules (6, 7)

<table>
<thead>
<tr>
<th>Molecule</th>
<th>( \Delta E_{\text{ONIOM}} \text{ (kJ/mol)} )</th>
<th>( \Delta E_{\text{ONIOM}} \text{ (kJ/mol)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1.611,037.86</td>
<td>-388.70</td>
</tr>
<tr>
<td>2</td>
<td>-1.610,649.16</td>
<td>-390.00</td>
</tr>
<tr>
<td>3</td>
<td>-1.546,401.00</td>
<td>-1094.68</td>
</tr>
<tr>
<td>4</td>
<td>-1.455,559.00</td>
<td>-905.84</td>
</tr>
<tr>
<td>5</td>
<td>-1.513,448.00</td>
<td>-910.72</td>
</tr>
<tr>
<td>6</td>
<td>-1.434,180.00</td>
<td>-799.66</td>
</tr>
<tr>
<td>7</td>
<td>-1.443,054.00</td>
<td>-930.00</td>
</tr>
</tbody>
</table>

\( \Delta E_{\text{ONIOM}} \) increases: 2 < 1 < 3 < 4 < 5 < 6 < 7

Two different geometries are most stable: the first one with coumarin moiety (head) located inside CD cavity and the second with the celllobio-side (tail) moiety resides inside CD cavity. The latter is more stable by about 20 kJ/mol.

\( \Delta E_{\text{ONIOM}} \) increases: 2 < 1 < 3 < 4 < 5 < 6 < 7

Two different geometries are most stable: the first one with coumarin moiety (head) located inside CD cavity and the second with the celllobio-side (tail) moiety resides inside CD cavity. The latter is more stable by about 20 kJ/mol.
Table 5  Effect of solvent polarity on the thermal stability of molecule (6) [4-MU-β-xylopyranoside (β-xylosidase substrate)] complex with β-CD

<table>
<thead>
<tr>
<th>Molecule</th>
<th>(High, model) (a.u.)</th>
<th>(Low, real) (a.u.)</th>
<th>(Low, model) (a.u.)</th>
<th>(E_{\text{ONIOM}}) (kJ/mol)</th>
<th>(\Delta E_{\text{ONIOM}}) (kJ/mol) relative to gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas</td>
<td>−1101.6659440</td>
<td>−2.7085319</td>
<td>−0.3569707</td>
<td>−2,898,597.96</td>
<td>−</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>−1101.6737106</td>
<td>−2.7327139</td>
<td>−0.3628978</td>
<td>−2,898,666.28</td>
<td>−68.32</td>
</tr>
<tr>
<td>Water</td>
<td>−1101.6821033</td>
<td>−2.7585906</td>
<td>−0.3692911</td>
<td>−2,898,739.47</td>
<td>−141.51</td>
</tr>
</tbody>
</table>

# oniom(B3LYP/3-21G:PM3) scrf = (cpcm, solvent = water, oniompcm = x)  \(\text{ONIOMPCM}=x\) performs an ONIOM calculation in solution [42] according to the scheme \(x\). Thus, the reaction field is computed separately in each sub-calculation always using the cavity of the real-system. This is the default if ONIOM and SCRF are specified (available for energies, optimizations and frequencies).

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33. Spartan'16: Parallel, Wavefunction Inc., USA

34. Spartan 16: Parallel, Wavefunction Inc., USA


