Supporting Information

Study of the Complexation of Fisetin with Cyclodextrins

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Applications of Quantum Mechanical Methods.

All the calculations were carried out for single structures or taking associations between one Fis molecule and one molecule of β-CD into account. The ground state of the isolated Fis was initially optimized using the Hartree-Fock Theory (UHF). Afterwards, the DFT method based on the B3LYP hybrid functional was employed for comparison purpose. The Configuration Interaction Singles *ab initio* method was used for the optimization of the excited singlet S_1 state. TD-DFT (B3LYP hybrid functional) calculations were performed to explore the parameter related to the Fis S_1 state and the corresponding deprotonated molecule. UHF, CI-Singles, and DFT calculations were accomplished using the basis set 3-21G** and the software Gaussian 03W (Rev. B05, Version 6), together with GaussView 03[1].

The NMR spectrum of Fis in its ground state was obtained by application of DFT based on the MPW1PW91 functional and the 6-311G* basis set[2]. The hydrogen chemical shifts were calculated by taking the TMS shielding constant (31.8821, calculated using B3LYP/6-311G* GIAO, "Gauge-Independent Atomic Orbital") and the key-words NMR = IGAIM1.

The structures of β-CD and of the Fis-β-CD complex were initially optimized using the SAM1 (UHF) semi-empirical method, with a quadratically convergent SCF calculation, based on the AMPAC software[3]. Energy minimization was obtained by employing the TRUSTG software, with gradient equal to 0.001. Thermodynamic parameters were gained including the keywords FORCE and THERMO as input.

Fis Intramolecular Excited State Proton Transfer (IESPT).

The theoretical results suggest that, geometrically speaking, the difference between Fis in the S_0 and S_1 states is very subtle and is located in the part of the molecule where Fis and its tautomer exist in equilibrium. However, in the ground state, the equilibrium between Fis and its tautomer is prevented by a high energetic barrier. In the excited state, there is a change in the Fis electronic density. For Fis at S_0, the planarity between the phenyl ring and the chromone is maintained (dihedral angle between carbons C(4)C(3)C'(1')C'(2') = 0.000 ° - Scheme 1, Fig. S1). As for the tautomer, the nearby carbonyl group (C(4)O) provokes a small deviation from this planarity (dihedral angle = -0.004 °) and the estimated dipole moment decreases to 1.8806 D. So, in the excited state, the equilibrium between the Fis normal structure and its tautomer becomes feasible because the energetic barrier is lower.
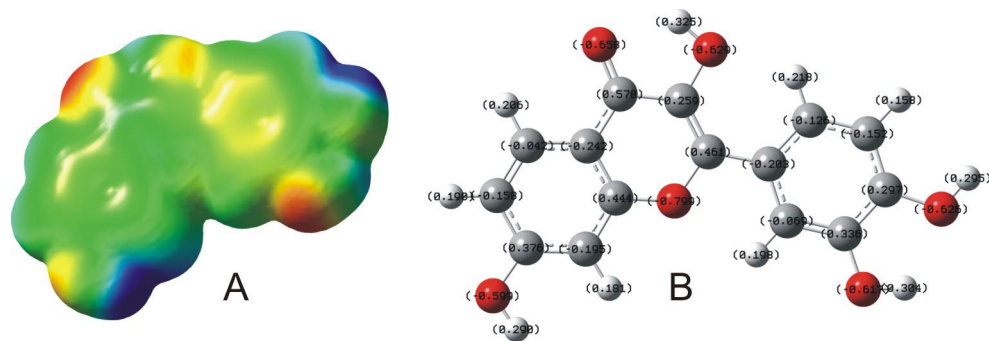

Figure S1. (A) Fis electronic density and (B) Mulliken charges.

Quantum-Mechanics Calculations for the Fis-β-CD Complex.

The SAM1 semi-empirical method was chosen to describe the larger systems (β-CD and inclusion complexes). Its characteristic attributes of *ab initio* methods[3] gives an improved formalism over its precursors (MNDI and AM1). The large number of atoms involved, 178 in the case of the inclusion complex, led to this choice. Also, in order to reduce computational costs, the theoretical data did not consider the possible interactions between the compounds and the solvent.

Our optimization of the structure of the inclusion complex considered both ways of entry of the flavonoid into β-CD: either through chromone (A) or via the phenyl group (B) of the Fis molecule (Scheme 1). Fis insertion was performed both via: (i) "construction" and later optimization of the inclusion structure and (ii) by employing a reaction path procedure to evaluate Fis entry into β-CD. During the latter calculation, up to 47 structures were optimized, and they corresponded to the steps involved in the formation of the inclusion complex. Deprotonated Fis, simulating a pH *ca.* 12, was also calculated under the same conditions above. Theoretical thermodynamic parameters were determined and are depicted in Table S1.

Table S1. Theoretical thermodynamic parameters for the complexation of Fis with β-CD, derived from the SAM1 semi-empirical model.

Thermodynamic Parameter	Theoretical (SAM1) (Standard values at 25 °C)
ΔH (kJ.mol^{-1})	-48.95 (phenyl)
	-44.43 (chromone)
	-68.41 (phenyl, pH 12)
ΔS (J.mol^{-1}.K^{-1})	-186 (phenyl)
	-127 (chromone)
	-165 (phenyl, pH 12)
$\Delta G25$ (kJ.mol^{-1})	+6.5 up to +18.2 (phenyl)

-6.6 (chromone)

-19.3 (phenyl, pH 12)

The theoretical data of Table S1 suggest the coexistence of the two forms of inclusion complex, with preferable inclusion via the chromone. However, calculations showed that when Fis is inserted through the phenyl group, the hydroxyl protons 3' and 4' can form intermolecular hydrogen bonds with the O atom in position 6 of the β-CD molecule, with estimated bond lengths of 1.81 and 1.74 Å, respectively. The hydroxyl oxygen in position 3' is also capable of forming an intermolecular hydrogen bond with β-CD, with an evaluated bond length of 1.89 Å. In addition, the hydrogens of OH 3' and OH 4' form intramolecular bonds with the oxygen of the neighbor hydroxyl, with calculated bond lengths of 2.19 and 1.89 Å, respectively.

The formation of a complex via chromone entry gives rise to the formation of only one intermolecular hydrogen bond between the hydrogen of the Fis OH 7 and the OH group in position 6 of β-CD, with an estimated bond length of 1.72 Å. In this case, the calculations from the reaction path procedure also suggest two structures for the inclusion complex (Fig. S2), separated by an energy barrier of ca. 12.2 kJ. The first structure has two hydrogen bonds (1.86 and 1.77 Å) and is more stable, while the other displays only one hydrogen bond (1.8 Å).
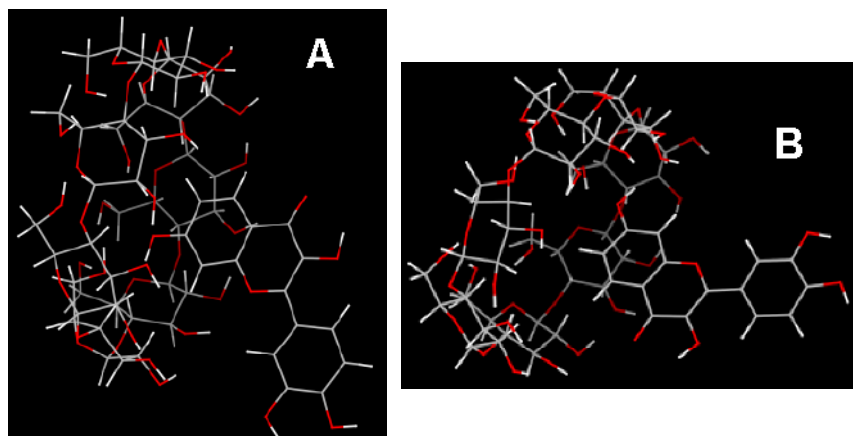
Figure S2. Data obtained by applying the SAM1 semi-empirical model to the study of the incorporation of Fis into β-CD. Evaluation of the entry via chromone presenting (A) two or (B) only one hydrogen bond.

So, considering only the number of hydrogen bonds formed, it should be expected that the inclusion complex should be predominantly based on the phenyl group entry. In addition, in the study carried out by the reaction path method, the entry via the chromone ring of the molecule points out to many energy barriers for Fis entry. They result from the formation of several intermediates produced by the hydrogen bond between the OH 7 of the chromone and the OH groups in the entrance of the β-CD cavity. This reduces the possibility of Fis inclusion via the chromone entry. On the other hand, for the phenyl entry, the calculations point to one product only, without intermediates. From these results, we believe that the preferential entry is through the phenyl ring, although Table S1 indicates the opposite.

It is worth to mention that the hydrogen bond lengths estimated by theoretical calculations are short. However, in a recent work, it has been shown that the introduction of interaction with solvents in the calculations tend to approximate the theoretically estimated values to the typical ones attributed to hydrogen bonds[4].

For deprotonated Fis, simulating a condition of pH *ca.* 12, both types of entry bring about a distortion in the β-CD structure (Fig. S3). However, the ΔG^{o}_{R} value for the inclusion via phenyl (Table S1) suggests a strong stabilization of the formed system when compared with neutral Fis. The optimized structure for this complex shows there are strong interactions between the phenoxide and OH groups present in the entrance of the β-CD cavity, due to the formation of many hydrogen bonds, one of them resulting from the sharing of a proton. Two typical intermolecular hydrogen bonds (both 1.64 Å) and one intramolecular hydrogen bond involving the phenoxide anion and the neighbor OH group over the phenyl group (2.07 Å) can be observed. This OH group also interacts with β-CD through its oxygen,

forming one hydrogen bond with a length of 1.77 Å When a proton is shared, the β-CD C-H bond length increases to 1.14 Å, and the hydrogen turns up as an isolated atom shared by the β-CD and the phenoxide anion. The distance from this hydrogen to the phenoxide is 1.90 Å.
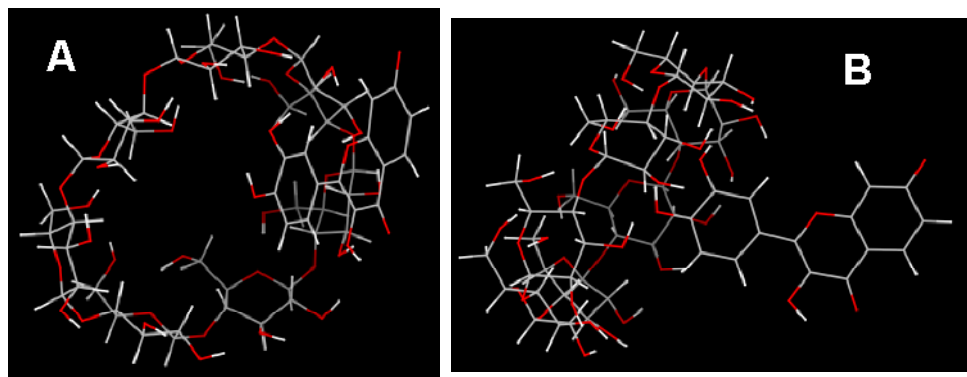

Figure S3. Data evaluated from the application of the SAM1 semi-empirical model to the study of the inclusion of deprotonated Fis to β-CD via the phenyl group (A) over and (B) lateral vision.

It should be borne in mind that these results were obtained from calculations performed on isolated molecules, in the absence of any interaction with solvent molecules. Certainly, a study involving 'Simulated Annealing" to estimate the more stable conformations of the inclusion complexes, also including the participation of solvent molecules, could give more reliable thermodynamic parameters. The limitation of this study is the high computational cost.

NMR measurements.

For comparison purposes, the theoretical chemical shifts obtained by molecular modeling for Fis are also presented.

Table S2. ^{1}H NMR chemical shifts δ (ppm) and coupling constants J (Hz) for Fis in different media.

Fis	$D_2O/NH_{3(g)}$ (pD ~ 11.5) Sample 1	$D_2O/NH_{3(g)}$ (pD ~ 11.5) Sample 2	Theoretical data[a]	CD_3OD
H6	6.74, dd, $J_{6,5}$ = 8.95 $J_{6,8}$ = 2.27	6.24, dd, [b]$J_{6,5}$ = 8.69 $J_{6,8}$ = 2.38	6.69	6.9-6.94, m
H5	7.83, d, $J_{5,6}$ = 8.95	7.58, d, $J_{5,6}$ = 8.69	7.91	8.00, d, $J_{5,6}$ = 8
H8	6.65, d, $J_{8,6}$ = 2.23	6.11, d, $J_{8,6}$ = 2.35	6.03	6.9-6.94, m
H2'	7.65, d, $J_{2',6'}$ = 2.33	7.31 – 7.29, m	7.16	7.79, d, $J_{2',6'}$ = 4
H6'	7.60, dd, $J_{6',5'}$ = 8.35 $J_{6',2'}$ = 2.36	7.31 – 7.29, m	7.43	7.69, dd, $J_{6',5'}$ = 4 $J_{6',2'}$ = 8
H5'	6.82, d, $J_{5',6'}$ = 8.39	6.65, d, $J_{5',6'}$ = 8,46	6.42	6.9-6.94, m
OH7	n. o.	n. o.[c]	2.04	n. o.
OH3			3.33	
OH3'			2.34	
OH4'			1.33	

[a] DFT MPW1PW91, with B3LYP/6-311G* GIAO for the calculation of the TMS shielding constant;

[b] d = doublet, dd = double doublet, m = multiplet;

[c] n.o.: signal not observed.

Table S3. ^{1}H NMR chemical shifts δ (ppm) for Fis, β-CD, and mixtures of host and guest in $D_2O/NH_{3(g)}$ solutions.

	Fis	β-CD	Fis - β-CD complex (12 mM Fis: 15 mM β-CD)
H6	6.24		6.23
H5	7.58		7.59

H8	6.11		6.12
H2'	7.31 – 7.29		7.31 – 7.29
H6'	7.31 – 7.29		7.31 – 7.29
H5'	6.65		6.63
H1		5.09	5.07
H2		3.67	3.64
H3		3.99	3.97
H4		3.61	3.58
H5		3.88	3.84
H6		3.91	3.87

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