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Molecular dynamic simulation and docking energy to forecast the stability of βCyD-complexes in water solution

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Abstract

Establishment of molecular modelling able to simulate the behaviour of the βCyD-complexes in aqueous solution was studied by applying the fundamentals of classic mechanics. The existence of stable complexes was experimentally determined by the findings of ^{1}H NMR NOE (Nuclear Overhauser Effect) experiments in aqueous solution. A 'training set' of molecules was chosen to test the ability of the mathematic models to describe the behaviour of βCyD-complexes. A 'test set' group of molecules was defined to better confirm the validity of the proposed models. Based on the results, molecular modelling seems to be a useful tool for forecasting the behaviour of βCyD-complexes in water solution since the results of the mathematic models are close to the experimental findings obtained from NOE experiments.

Keywords: Molecular dynamic simulation; βCyclodextrin; ^{1}H NMR; Nuclear Overhauser effect

1. Introduction

In order to investigate molecular complexes with cyclodextrins (CyDs) it has been found that NMR NOE (Nuclear Overhauser Effect) and molecular modelling studies are very useful tools in obtaining direct evidence of the complexation and a better description of the supramolecular edifice. Molecular modelling investigations are generally associated to NMR studies because they represent a complementary method in rationalizing the experimental findings (Cabral Marquez et al., 1990; Amato et al., 1992; Mulinacci et al., 1993). The aim of the present study was to establish a molecular modelling able to simulate the behaviour of the βCyD-complexes in aqueous solution by applying the fundamentals of classic mechanics.

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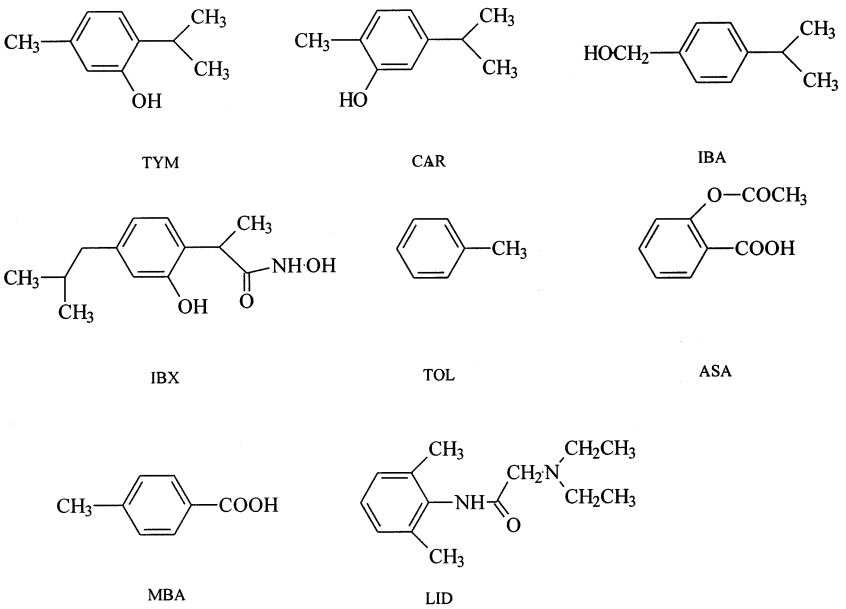

Fig. 1. 'Training set' group of molecules.

A 'training set' of eight molecules (Fig. 1) was chosen in order to test the ability of the models to describe the behaviour of the corresponding βCyD-complexes in aqueous media. Four of these compounds gave stable complexes in water: thymol (THY) ibuproxam (IBX), carvacrol (CAR) and *p*-isopropylbenzoic acid (IBA). The other compounds gave unstable complexes in water: toluene (TOL), lidocaine (LID), acetylsalicylic acid (ASA) and *p*-methylbenzoic acid (MBA).

A 'test set' group of molecules (Fig. 2) was defined to better confirm the validity of the proposed models. All the components of this group belong to the class of *p*-hydroxybenzoic acid esters: methyl (MHB), ethyl (EHB), propyl (PHB) and butyl (BHB) esters were investigated in particular. Among these, only the two esters EHB and PHB gave stable complexes in water at room temperature. The existence of the stable complexes was experimentally determined by the findings of ^{1}H NMR NOE experiments performed in aqueous solution.

2. Materials and methods

2.1. Materials

The β-cyclodextrin(βCyD) was purchased from Sigma (St. Louis MO) and used without any further purification. The guest molecules were purchased from Fluka Chemie AG (Buchs, Switzerland). The complexes were obtained by co-precipitation, adding equimolar concentrations of the guest to saturated solutions of βCyD in distilled water, after stirring for 24 h. The precipitates were filtered, dried and utilized for the NMR experiments.

2.2. Methods

2.2.1. ^{1}H NMR studies

Chemical shifts were measured relative to the peak of the solvent D_2O (4.74 ppm) with a Bruker AMX 600 at 600 MHz in a Fourier transform mode. The NOE experiments were performed on

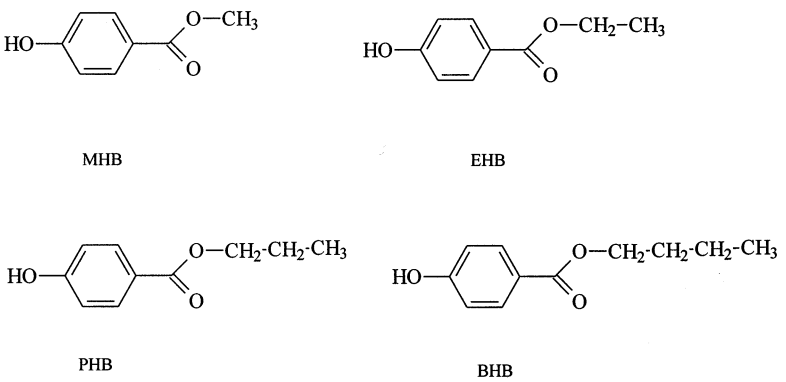

Fig. 2. 'Test set' group of molecules.

a Varian Gemini 200 at 200 MHz with a Varian NOE DIFF program, version 6.3A. All spectra were recorded with a 5 mm tube at the probe temperature (25°C) in D_2O, without degassing, near to the saturation point.

The NOE measurements were performed in 'steady state' conditions. For each sample a value of the presaturation time was at least four times higher than the average relaxation time of the presaturated protons.

2.2.2. Computer graphics

For each βCyD-complex, the conformational energy was evaluated applying the Discover program, version 2.9.7., from Biosym Technologies (San Diego, CA) (Biosym/MSI, 1995), run on the Personal Iris from Silicon Graphics. The force field calculations were performed using the AMBER method (Weiner et al., 1984, 1986). In addition, the original AMBER method was modified adding both specific parameters for carbohydrates according to Homans (1990) and parameters for glycoproteins according to Woods et al. (1995). Parameters applied for the dynamic simulations were: temperature = 300 K; equilibration time = 1 ps; dynamic simulation at 300 K = 100 ps (100000 steps); history file output frequency = 1 ps; timestep = 1 fs.

For all the methods the dielectric constant parameter (D) was applied with a value of 1.

In docking, the interaction energy is computed by summing the energy contributions between all atoms of the two molecules forming the complex. The contribution between atoms interacting with other atoms of the same molecule is ignored. The docking energy is calculated by the following equation:

$$\text{Energy}_{\text{interaction}} = \Sigma_i \Sigma_j (A/r_{ij}^{12} - B_{ij}/r_{ij}^{6} + q_i q_j/\epsilon r_{ij})$$

The interaction between water and guest molecules was simulated by the calculation of the docking energy between a water volume of 8000 angstroms^3 (corresponding to a cube with a side of 20 angstroms) and the guest molecule placed into the cavity of this cube. All water molecules constituting the cube have been considered as the one and only macromolecule for the docking energy calculation. A cut off of 10 angstroms has been applied for this calculation and all findings represent the interactions between the 'aqueous macromolecule' and the guest molecule.

For all guest molecules dummy atoms were defined to represent the protons of the methyl, ethyl, isopropyl groups (Mulinacci et al., 1996). Evaluation of the intermolecular distance between the atoms of the guest molecules and the H3 and H5 of βCyD was performed using the dummy atoms described above and the real protons of βCyD.

3. Results and discussion

The efficacy of the models to simulate the behaviour of the βCyD-complexes in water was verified by comparing the NOE findings with the

molecular simulation data. In other words, the capability of the model to classify each complex in the correct group (e.g. stable complexes (S) or unstable complexes (U)) was studied. The experimental data, specifically ^{1}H NMR NOE measurements, were used as the main key for verification and evaluation of the complexes' stability in water. A complex was considered to be stable if it showed an Overhauser effect on the H3 and/or H5 protons of βCyD after presaturation of specific protons of the guest molecule.

3.1. ^{1}H NMR NOE findings

It is known that the analysis of intermolecular NOE represents a powerful tool in investigating the geometry of the inclusion βCyD-complexes in solution (Redenti et al., 1992). In fact NOE regards the dipolar interactions between protons as spatially close (<4 angstroms), therefore it provides useful information about the structure of the supramolecular system in solution.

For investigating the conformational structure of the molecules in solution, a NOE monodimensional experiment was chosen for this study (Neuhaus and Williamson, 1989). The low radio frequency of the available instrument (200 Mhz) was also a determinant.

Among the protons belonging to the guest molecule, only those showing a chemical shift far enough from the βCyD proton signals were presaturated in order to obtain unambiguous results. After presaturation, the increase of the signals due to the NOE was measured for the H3 and H5 inner protons of the βCyD cavity. A NOE DIFF program was used to directly obtain a spectrum showing only the increase of the signals.

Table 1 shows as the percent of signal increase related to the NOE increments for the guest compounds belonging to both the 'training set' and 'test set' groups of molecules.

3.2. Calculation of the conformational energy for βCyD

The conformational energies of the βCyD-complexes were evaluated applying a method of molecular mechanics where the force field calculations were carried out using the AMBER method (Weiner et al., 1984, 1986). In addition, the original AMBER method was modified adding both specific parameters for carbohydrates according to Homans (1990) and parameters for glycoproteins according to Woods et al. (1995). The Homans' parameters were applied to better describe either the atoms of the pyranosidic ring or other atoms bound to the ring, while the Woods' parameters were inserted to evaluate the contribution of the anomeric carbon and oxygen atoms.

To simulate the βCyD molecular behaviour, the following four methods based on AMBER were applied:

1. The original AMBER force field (A)
2. The AMBER modified using Homans (AH)
3. The AMBER modified using Homans and Wood (AW)
4. The AMBER modified using Homans and using Woods parameter only for the non-bond interactions (AHW).

The minimum value of the dielectric constant (D) was imposed for all the methods because it was reasonable to suppose only a weak effect of the water molecules around the edge of the hydrophobic cavity of βCyD.

For the βCyD-complexes, evaluation of the stability of the supramolecular system was corre-

Table 1
Percent of NOE increments measured among the studied βCyD-complexes

Guest	Guest protons	% NOE increments for H βCyD	
		H3 βCyD	H5 βCyD
TYM	H1	2.1	0.0
	H6	0.0	0.2
CAR	H1	1.3	0.0
	H6	0.0	0.0
IBA	H1	1.1	0.0
IBX	H1	0.8	0.0
	H9	0.0	0.2
EHB	H1,2	0.9	0.0
	H3,4	0.2	0.0
	H6	0.0	0.0
PHB	H1,2	0.4	0.0
	H3,4	0.5	0.0
	H6	0.0	0.0

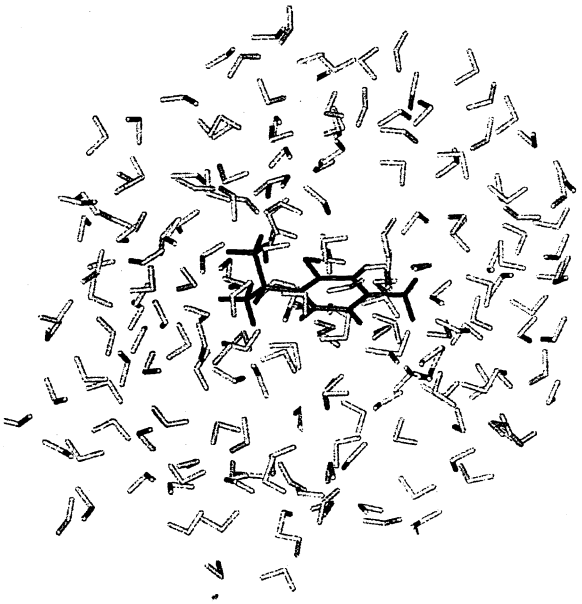

Fig. 3. Spatial conformation after minimization of the hypothesized complex thymol–H_2O.

lated to the value of the docking energy between the guest molecules and the βCyD. The docking energy was considered as a sum of Van der Waals' and electrostatic energies among all the atoms of the complex. This parameter represents the gain of potential energy due to interactions between the molecules forming the complex with respect to the sum of the energy of each molecule in a free state.The contribution due to non-bond interactions among the atoms of the same molecule has been omitted.

To better evaluate the stability of the βCyD-complex with respect to the guest–H_2O complex, the docking energy value between the guest molecule and the water was also calculated. The interaction between water and guest molecules was simulated by the calculation of the docking energy between a 'water cube' and the guest molecule placed into the cavity of this cube. For the docking energy calculation, the cube was considered as the one and only macromolecule and all findings represent the interactions between the 'aqueous macromolecule' and the guest molecule. It is interesting to note how after the energy minimization, the 'water cube' assumed a spheric shape as reported in Fig. 3.

Depending on either the asymmetry of the investigated compounds or of the βCyD molecule, it was necessary to hypothesize about the existence of two distinct complexes with different orientations for each guest molecule. The comparison between the two docking energy values can be a useful tool to verify the stability of the complexes. An example of the two reference structures is shown in Fig. 4 for the thymol βCyD-complexes.

The docking energy obtained at 0 K is not able to give realistic results because at this temperature the atoms are considered to be motionless in a fixed spatial position. Therefore, dynamic simulations at 300 K were performed to obtain docking energy values closer to the experimental conditions. However at 300 K a large number of different conformations can be hypothesized for each complex and to obtain representative values of the docking energy it was necessary to average the results derived from a large enough number of random conformations. To reach this goal, dynamic simulations, using the Discover program, were performed.

First it was necessary to choose the right simulation time, which had a wide enough range to permit registration of all the events related to the movements of the considered atoms. Considering both the appropriate simulation time and the available computer time, a total simulation time of 100 ps for all the studied structures was fixed.

Each dynamic simulation was constituted by 1×10^5 different conformations but only 100 of these were stored: one every 1 ps. The stability of the structures was better in the range from 10 to 100 ps because the variance of docking energy values was inferior with respect to that obtained during the first 9 ps of the dynamic simulation. Therefore, for each complex the average value of its docking energy was obtained selecting only 91 structural conformations collected in the time range 10–100 ps. For all the force field methods (A, AH, AW and AHW) the docking energy values were obtained applying the same dynamic parameters.

In Table 2 the average values of docking energy for all complexes belonging to the 'training set'

Table 2
Average values of docking energy for the complexes of the 'training set' group of molecules, evaluated at 300 K and obtained applying the different force field methods based on AMBER and modified AMBER

Guest	Com.	Guest–βCyD								Guest–H_2O		Experimental classification (NOE)[a]
		AMBER		AMBER Homans		AMBER Woods		AMBER Homans-Woods				
		Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.	
THY	1	−14.4	3.1	−12.8	1.9	−12.6	3.1	−11.4	2.2	−16.8	5.3	S
	2	−9.6	5.1	−12.9	1.7	−11.0	2.8	−10.6	4.2			
TOL	1	−11.5	1.5	−9.8	1.1	−10.6	1.2	−9.0	1.3	−18.5	1.5	U
	2	−10.9	1.2	−9.9	1.1	−0.5	1.2	−8.9	1.4			
IBX	1	−19.0	2.9	−16.6	2.4	−13.3	3.3	−14.0	3.2	−23.9	6.1	S
	2	−17.4	3.2	−15.7	2.9	−14.8	2.6	−14.9	2.9			
LID	1	−18.6	3.0	−14.8	2.7	−14.5	3.4	−12.8	3.4	−28.5	4.7	U
	2	−18.8	2.9	−18.2	2.9	−17.6	3.2	−13.1	2.7			
CAR	1	−13.4	4.1	−13.4	3.1	−12.8	2.7	−12.8	2.8	−16.2	5.2	S
	2	−6.8	4.8	−12.8	2.0	−10.0	4.1	−12.1	2.0			
IBA	1	−10.9	4.2	−12.8	2.5	−12.3	2.4	−11.9	2.7	−18.3	5.1	S
	2	−12.8	3.2	−12.6	2.4	−8.0	3.5	−8.8	4.6			
ASA	1	−12.3	5.8	−16.9	2.5	−13.7	2.9	−13.8	3.0	−21.6	5.6	U
	2	−12.8	3.2	−14.7	3.4	−12.4	3.1	−10.1	3.6			
MBA	1	−8.1	6.2	−11.5	2.9	−9.6	2.7	−11.8	2.7	−19.7	5.0	U
	2	−11.3	3.6	−12.0	2.5	−8.3	4.9	−11.4	2.2			

[a] S for stable, and U for unstable complexes.

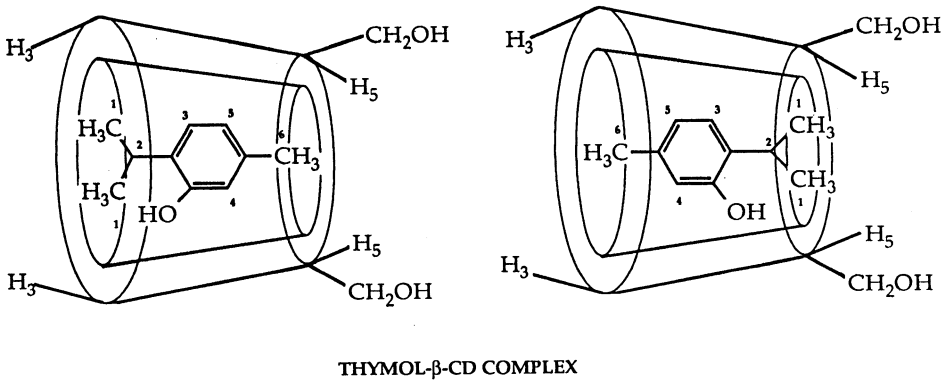

Fig. 4. Two possible conformations of thymol–βCyD complex.

group of molecules, evaluated at 300 K and obtained applying the different force field methods based on AMBER and modified AMBER, are reported.

3.3. Evaluation of the best simulation model

Discriminant analysis was applied to evaluate the accuracy of the four simulation models. The variables used in this analysis represent the best average values of the docking energy for the two conformers of every βCyD complex of the 'training set' group and the best average value of the docking energy for the complex with the 'water cube'. The findings of the NOE experiments were applied as classification key to divide the complexes as stable or unstable in aqueous solution. In other words, every NOE involving H3 or H5 βCyD protons was taken as discriminant parameter to classify the complex as stable (S) or unstable (U).

The discriminant analysis applied to the docking energy values for either guest βCyD-complex or guest H_2O–complex is sufficient since correct because only these data are characteristic parameters for each complex. In fact, observing the equilibrium

$$\underset{1}{\text{Guest}-H_2O} + \underset{2}{\beta\text{CyD}-H_2O} \rightarrow \underset{3}{\text{Guest}-\beta\text{CyD}} + \underset{4}{H_2O-H_2O}$$

it appears evident that terms 2 and 4 are the same for all the complexes and only terms 1 and 3 are specific of each complex.

The Fisher's linear classification function coefficients either for S or U complexes are reported in Table 3. Also in Table 3 the main statistic parameters for each discriminant function, calculated with the various force field methods for the 'training set' group of molecules, are reported. Observing the probability values (P) and the percentage values of the correctly classified cases, it appears evident how the unmodified force field AMBER (A) represents the best simulation model. Fig. 5 shows the scatterplot of the 'training set' group of molecules. It appears evident that the AMBER method is able to correctly divide the stable from the unstable complexes.

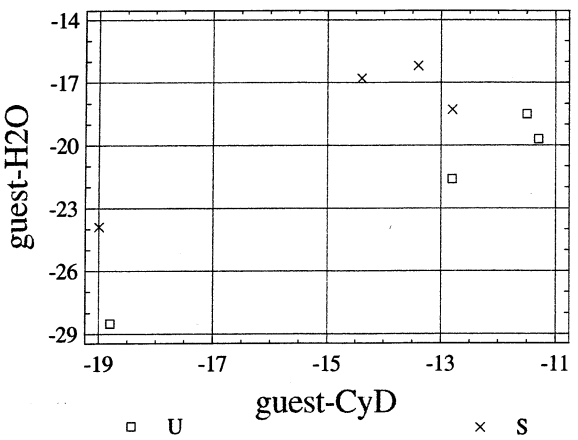

Fig. 5. Scatterplot of the 'training set' group of molecules, obtained with force-field Amber. U, unstable complexes; S, stable complexes.

Table 3
Discriminant analysis: the main statistical parameters for each discriminant function, calculated with the various force field methods for the 'training set' group of molecules

Force-field	Canonical correlation	*P*-Value	Classification function						Percent of correctly classified cases
			Coefficients for stable complex			Coefficients for unstable complex			
			g–βCyD	g–H_2O	Constant	g–βCyD	g–H_2O	Constant	
A	0.922	0.009	−0.72	−0.62	−11.85	3.65	−4.14	−21.55	100
AH	0.620	0.297	−0.67	−0.73	−12.23	0.005	−1.37	−15.77	75
AW	0.739	0.139	−1.33	−0.39	−13.04	0.026	−1.38	−15.77	87.5
AHW	0.688	0.201	−3.81	0.07	−24.34	−2.74	−0.48	−22.31	87.5

g, guest molecule.

Table 4
Evaluation of 'test set' group of molecules by force-field AMBER

Guest	Complex	Guest–βCyD		Guest–H_2O		Classification function values		Predicted[a] classification	Experimental classification (NOE)[a]
		Average	S.D.	Average	S.D.	Stable	Unstable		
MHB	1	−11.3	4.2	−17.4	5.8	7.07	9.24	U	U
	2	−12.2	3.7			7.72	5.96	S	
EHB	1	−10.0	4.2	−6.8	5.9	−0.43	−29.85	S	S
	2	−12.7	3.8			1.51	−39.71	S	
PHB	1	−12.7	4.2	−13.2	7.1	5.48	−13.21	S	S
	2	−13.8	3.4			6.27	−17.22	S	
BHB	1	−14.2	4.0	−21.3	6.2	11.58	14.85	U	U
	2	−15.8	2.9			12.73	9.01	S	

[a] S for stable, and U for unstable complex.

Table 5
Evaluation of 'test set' group of molecules by force-field AMBER-Woods

Guest	Complex	Guest–βCyD		Guest–H_2O		Classification function values		Predicted[a] classification	Experimental classification (NOE)[a]
		Average	S.D.	Average	S.D.	Stable	Unstable		
MHB	1	−12.0	2.6	−17.4	5.8	9.71	7.93	S	U
	2	−12.3	2.1			10.11	7.92	S	
EHB	1	−12.1	3.1	−6.8	5.9	5.71	−6.70	S	S
	2	−13.4	2.2			7.43	−6.73	S	
PHB	1	−13.1	2.6	−13.2	7.1	9.54	2.11	S	S
	2	−11.0	4.9			6.74	2.16	S	
BHB	1	−13.0	3.4	−21.3	6.2	12.56	13.29	U	U
	2	−12.7	3.1			12.16	13.29	U	

[a] S for stable, and U for unstable complex.

The AMBER-Woods (AW) method also seems to give acceptable results with a P of 0.139 and 87.5% of correctly classified cases.

The 'test set' group of molecules were taken as reference to evaluate the reliability of the two prediction models (A and AW) showing the best degree of fitting. Classification functions were used to divide the molecules of 'test set' in two groups forming, respectively, S and U complexes, with the function that yielded the largest value for an 'observation' representing the predicted group. The classification function was calculated for the two possible isomers of each complex of the 'test set ' group. In Tables 4 and 5, the evaluations of 'test set' group by force field A and by force field AW are reported, respectively.

According to the experimental classification (NOE data), the real stable complexes of the 'test set' were also predicted as stable by the simulation model A. It is interesting to note that for each isomer the differences between the minimum value, relating to U complexes, and the maximum value related to S complexes, as calculated by the classification function are both very high.

For U complexes like MHB and BHB, the simulations performed with the force field A showed that only one of the two possible conformers appeared to be unstable. On the other hand, with the force field AW, both the conformers of MHB are incorrectly classified as stable while for BHB both the conformers result as unstable (Table 5).

Concerning the experimentally unstable complexes (NOE data), differences between the values of the classification functions for both the U and S complexes appear to be very little.This means that the prediction can be considered uncertain.

In light of the obtained results, molecular mechanics seems to be a useful tool for forecasting the behaviour of βCyD-complexes in solution since the results of the mathematic models are sufficiently in agreement with the experimental findings derived from NOE experiments.

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