NMR spectroscopic evidence and molecular dynamics studies on inclusion and non-inclusion phenomena between β-cyclodextrin and new anti-Alzheimer's drugs tacrine (CI-970), velnacrine (HP-029) and suronacrine (HP-128)

Maria E. Amato,*[a] Kenny B. Lipkowitz,[b] Giuseppe M. Lombardo[c] and Giuseppe C. Pappalardo[c]

[a] *Dipartimento di Scienze Chimiche, Università di Catania, Viale A. Doria 6, 95125 Catania, Italy*

[b] *Department of Chemistry, Indiana University–Purdue University at Indianapolis, Indianapolis, IN 46202-3274, USA*

[c] *Dipartimento di Scienze Chimiche, II Cattedra di Chimica Generale, Facoltà di Farmacia, Università di Catania, Viale A. Doria 6, 95125 Catania, Italy*

The formation of supramolecular complexes of β-CD with new generation anti-Alzheimer's agents of the 1,2,3,4-tetrahydro-9-aminoacridine family (tacrine hydrochloride, 1, velnacrine maleate, 2 and suronacrine maleate, 3), has been studied using molecular dynamics (MD) simulations both *in vacuo* and with a water sphere. High-field NMR experiments for 1 and 2 gave experimental evidence of their effective non-inclusion. However, earlier NMR studies demonstrated inclusion of 3 on the basis of the observed upfield shifts of the 3-H and 5-H protons of the β-CD cavity.

MD calculations performed for molecules *in vacuo* gave uncertain results that evidenced failure of this model to reproduce consistently the set of the three experiments. Such a failure was overcome by including explicitly water molecules in the model, thus showing that an oversimplified model for modelling complexes of charged molecules is susceptible to incorrect conclusions.

The calculations performed for cations 1, 2 and 3 in the water sphere also showed that 1 and 2 are more hydrophilic than 3. This suggests that the relative strength of the solvation and of the complex formation constants favours complexation of 3 instead of its non-inclusion, and *vice versa* in the cases of 1 and 2.

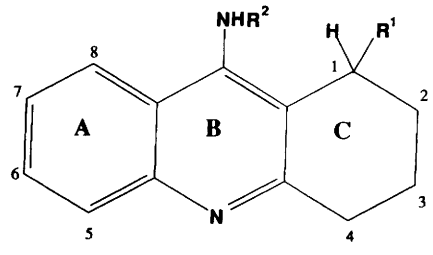
An important general aspect of β-CD supramolecular complexes of charged species was found to be controlled by the solvation effects that must be necessarily included to correctly model, in the absence of safe experimental information like that coming from NMR, inclusion or non-inclusion of a guest into the host.

Introduction

Cyclodextrins (CDs) are cyclic molecular encapsulators of a variety of organic and inorganic guest molecules and have been intensively investigated for their potential ability in drug delivery,[1] in molecular recognition[2] and in enzyme mimics for the substrate binding processes in the aqueous phase.[3]

A major advance in the use of CDs within the pharmaceutical industry has come from their application as vehicles for the delivery of a variety of very important drug classes.[1] Anti-Alzheimer's disease (AD) and cognition-activators[4] are two classes of drugs which present major problems in their use, *i.e.* bio-availability, toxicity and in some cases, low solubility. The efficacy of the new generation of anti-AD agents such as 1,2,3,4-tetrahydro-9-aminoacridine derivatives, in particular, 9-amino-1,2,3,4-tetrahydroacridine hydrochloride **1** (tacrine, CI-970),† (±)-9-amino-1,2,3,4-tetrahydroacridin-1-ol maleate **2** (velnacrine, HP-029)† and (±)-9-[(phenylmethyl)amino]-1,2,3,4-tetrahydroacridin-1-ol maleate **3** (suronacrine, HP-128)† has been fully established by human trials.[5–7] We thus considered native CDs as candidate host compounds for increasing oral bioavailability and decreasing the rate of distribution and/or elimination of these drugs.

Recently we have studied the complexation of racemic mixtures of **3** with CDs[8] using high-field NMR techniques and force field molecular modelling, which also evidenced enantiomeric discrimination. The present work, using NMR data and molecular dynamics methods, was aimed to study: (*i*) the CDs capability to complex the new anti-AD agents **1** and **2**, (*ii*) the extension by which the hydrophilic–lipophilic properties of the candidate guest molecules may influence the formation of the complexes.



Compound	R^1	R^2	
1	H	H	hydrochloride monohydrate
2	OH	H	hydrogenmaleate
3	OH	CH_2Ph	hydrogenmaleate

Scheme 1

† Through the whole paper the drugs coded CI-970, HP-029 and HP-128, will be named for brevity as **1**, **2** and **3** (Scheme 1), respectively.

Results of the molecular dynamics (MD) simulations performed in this study also were useful to highlight the general problem of the limitations and possible pitfalls of interpretive oversimplified models of host–guest complexes.

Experimental

Materials

Tacrine hydrochloride **1** (Cognex®) was supplied by Warner-Lambert. Samples of racemic velnacrine maleate **2** (Mentane®) and of racemic suronacrine maleate **3** were kindly provided by Hoechst-Russel. Cyclodextrins were commercial Fluka products used without further purification.

NMR spectroscopy

All experiments were performed at 500.13 MHz on a Bruker AMX-500 spectrometer in D_2O solution employing standard conditions. The probe temperature was 298 K and proton shifts were referenced to external TMS.

Complexation studies were performed on samples prepared by mixing equal volumes of D_2O stock solutions (10 mmol dm^{-3}) of guest and CDs. All the mixtures were freeze-dried twice from D_2O to minimize the HOD present in the aqueous samples due to the exchange of CD hydroxyl protons for deuterium.

The stoichiometry of the complex of **3** with β-CD was evaluated through NMR spectroscopic experiments by using the continuous variation method. The ^1H chemical shift data were determined from mixtures of **3** and β-CD in which the overall molar concentration was kept constant at 10 mmol dm^{-3} and the molar fraction of the two components was varied from 0 to 1. The corresponding job plots[9] for the β-CD cavity protons 3-H and 5-H were obtained using chemical shift data. The plots had highly symmetric shapes with a maximum at 0.5, thus indicating a 1 : 1 stoichiometry for the inclusion complex of **3** as well as the absence of contributions from other complexes.

Molecular dynamics procedure

The calculations were performed with the CHARMm force field[10] (CHARMm program, version 2.2, released by Molecular Simulations, Cambridge, UK) running on HP-710 workstation. All the minimizations were carried out using the ABNR (Adopted Basis Newton-Raphson) algorithm up to a gradient of 0.01 kcal mol^{-1} $Å^{-1}$. All the MD simulations in this study used the SHAKE algorithm on bonded hydrogen atoms and were performed at 298 K using a constant temperature algorithm (CPT CHARMm command). The thermal bath coupling constant and the integration time step were 5.0 and 0.002 ps, respectively.

The total charge for each of the cationic species **1**, **2** and **3** was set to +1. The counterions were considered to be fully solvated, solvent separated ions and not included in the calculations.‡

The atomic charges of each atom were set at the values obtained by fitting the molecular electrostatic potentials[11] (MEP) calculated through the MNDO method of MOPAC-6 package.[12]

The input coordinates for structure minimizations of the isolated molecules came from the X-ray crystal structures of the cationic guests[13] and β-CD,[14] 'cleaned up' by deleting other extraneous atoms. For all the calculations we used only the *R* enantiomer of both **2** and **3** molecules. This was because (*i*) previous studies on **3**[8] showed that for this guest the *R* enantiomer binds into the macrocycle more deeply than the *S* one and (*ii*) preliminary checks showed that results from parallel gas-phase simulations of *R* and *S* enantiomers of **2** did not differ.

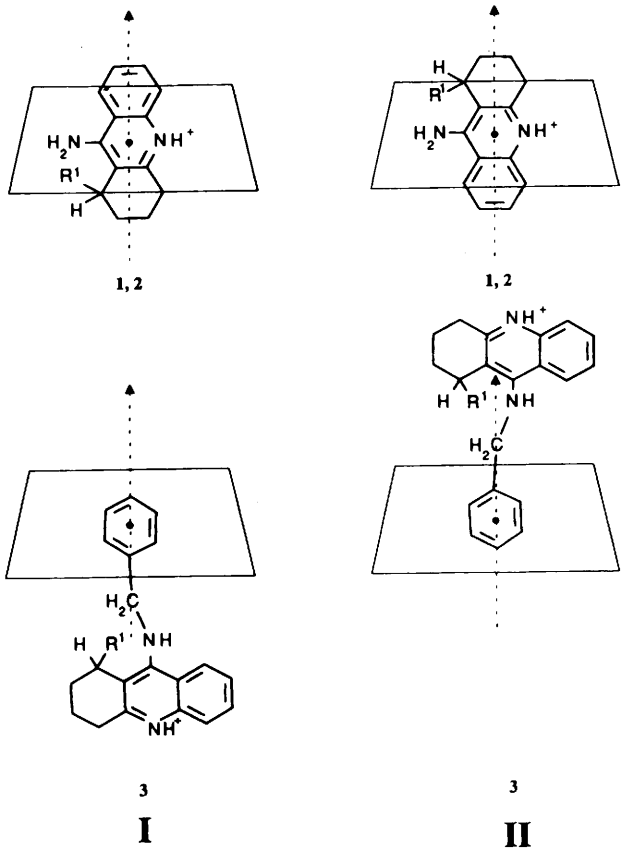


Fig. 1 Alignment scheme used for definition of the relative host–guest orientations **I** and **II**. The symmetry axis of β-CD is denoted by the dashed line; the arrow indicates the positive direction of this axis, whose origin is placed at the mass centre of the torus (marked by •). In the figure, the mass centres of the host and guest are set coincident before the minimization that produced the starting structures for dynamics. In the case of **3** the mass centre of β-CD and the geometric centre of the phenyl ring are overlapped in the structure to be minimized.

On the basis of steric considerations, the host and guest molecules **1** and **2** were accommodated in an axial orientation and aligned along one of their principal axes of inertia. Each guest **1** and **2** was embedded into the host up to coincidence of their mass centres. In the case of the complex of **3**, which is formed by inclusion of the guest's phenylmethyl group,[8] the starting structure was constructed by overlapping the β-CD mass centre and the phenyl geometric centre. The supramolecular edifice of each complex was built twice by changing the relative orientation (**I** and **II**) of the guest along the β-CD axis, as depicted in Fig. 1.

The complexes so obtained, after minimization, were submitted to MD simulations using distance-dependent relative permittivity ($\varepsilon \propto R$) and a cut-off radius of 15 Å for the non-bonded interactions *in vacuo*. Simulations in the gas phase were run for 400 ps (200 000 steps) and sampled every 100 steps.

The MD simulations were also carried out by considering explicitly the solvent molecules using the water three interaction sites model TIPS3P of the CHARMm package.

The protocol included immersion of every cation and host–guest complex, previously minimized in the gas phase, in the water sphere of diameter 30 Å, whose density was set at the experimental value (1 g cm^{-3}), and therefore containing 464 water molecules. The successive steps were (*i*) removal of water

‡ As expected on the basis of the chemical stucture of the cations **1**, **2** and **3**, the MNDO calculated atomic charges, for use in force field calculations, clearly denoted significant delocalization of the total charge of each cation. This fact in addition to dilution of the samples in the experiments (5 mmol dm^{-3}) should make more reasonable the assumption of very weak ionic interactions and, accordingly, of non-inclusion of the counterion in the calculations. Furthermore, in the above conditions the probability of finding a counterion within the adopted 30 Å diameter water sphere should be a minimum, so that the simulated model is the closest one to the natural conditions.

molecules from within a distance of 2.8 Å all around the solvated molecule to avoid overlapping of some solvent molecules with the solute and (*ii*) minimization of such solute–solvent system by relaxing only the water molecules while keeping the solute coordinates constrained. The starting structures for MD simulations were then obtained by successive minimization of the whole fully relaxed solute–solvent system. For the non-bonded interactions a distance-independent relative permittivity ($\varepsilon = 1$) was used and the cut-off radius was set to infinity. All the solvated systems were simulated for 120 ps (60 000 steps) with a sampling frequency of 100 steps. Every simulation was repeated at least four times to verify the reproducibility of the result.

It should be stated that an MD procedure aimed at simulating the inclusion process for a host–guest complex requires suitable initial conditions to attain a finite probability of effective embedding of the guest into the host. Simulation results in line with experimental evidence of inclusion can be produced by this procedure, but only for a limited percentage of trial MD runs. This is an intrinsic feature of the method, whereby a large number of MD runs will not bring to fruition the formation of an inclusion complex. It follows that, for a system in which the experiments denote lack of effective inclusion, the dynamics procedure we adopted, simulating extrusion instead of inclusion of the guest, is more justified and in order. In this case the experimental results can be described in a sufficient way by simulations that verify the stability of the host–guest complex or, alternatively, the permanent extrusion of the guest from the host during the whole evolution time. Accordingly, the starting supramolecular structures as obtained by energy minimizations, *in vacuo* as well as solvated, were assumed accessible structures whose stability was to be verified through dynamics. For this, in the protocol, the initial temperature was set to 0 K, to avoid imposition of privileged relative directions of the host and guest with respect to their mass centres. The system was therefore monitored from the initial step of the run including the thermal equilibration period.

Results and discussion

NMR Data

The assignments of the ^{1}H NMR spectra of the aqueous solution of **1** [δ_H 2.27 (2 H, m, 1-H, 1′-H), 1.85 (4 H, m, 2-H, 2′-H, 3-H, 3′-H), 2.68 (2 H, m, 4-H, 4′-H), 7.3 (1 H, dd, 5-H), 7.72 (1 H, dt, 6-H), 7.45 (1 H, dt, 7-H), 7.7 (1 H, dd, 8-H)] and **2** [δ_H 5.1 (1 H, t, 1-H), 2.2 (1 H, m, 2-H), 2.05 (3 H, m, 2′-H, 3-H, 3′-H), 3.12 (1 H, m, 4-H), 2.97 (1 H, m, 4′-H), 7.74 (1 H, dd, 5-H), 7.94 (1 H, dt, 6-H), 7.68 (1 H, dt, 7-H), 8.21 (1 H, dd, 8-H)] were made on the basis of chemical shift considerations supported by the previous assignment of the **3** spectrum.[8]

The upfield shift of the resonances of the internal cavity protons 3-H and 5-H of CDs observed in the ^{1}H NMR spectrum of a mixture of the host and guest with respect to the spectra of the pure compounds at the same concentration may be interpreted in terms of inclusional interaction of the two molecular systems.[15]

Careful inspection of the ^{1}H NMR spectra of the aqueous equimolar solution of **1** with α-, β- or γ-CD gave evidence of absolute lack of internal or external interaction between the two molecules. Actually, the spectrum of the mixture of **1** and CDs can be simply considered to be the superposition of the spectra of the two pure compounds at the same concentration.

Chemical shifts, splittings, as well as resolution, remained unchanged also in the spectra of an equimolar mixture of **2** and CDs, with respect to their pure solutions at the same concentration, thus denoting the lack of formation of inclusion complexes.

The complexation of **3** by β-CD was widely evidenced by the ^{1}H NMR spectrum of an equimolecular host–guest mixture when compared with the spectra of the pure components. In particular, the large upfield shifting of 3-H ($\Delta\delta = \delta_{free} - \delta_{mix} = 0.113$ and 5-H ($\Delta\delta = \delta_{free} - \delta_{mix} = 0.110$) was assessed and attributed to the inclusion of **3** in the host cavity. The 2D NMR-ROESY experiment previously reported by us[8] confirmed the formation of the complex and in addition gave unambiguous information on the geometry of the supramolecular edifice.

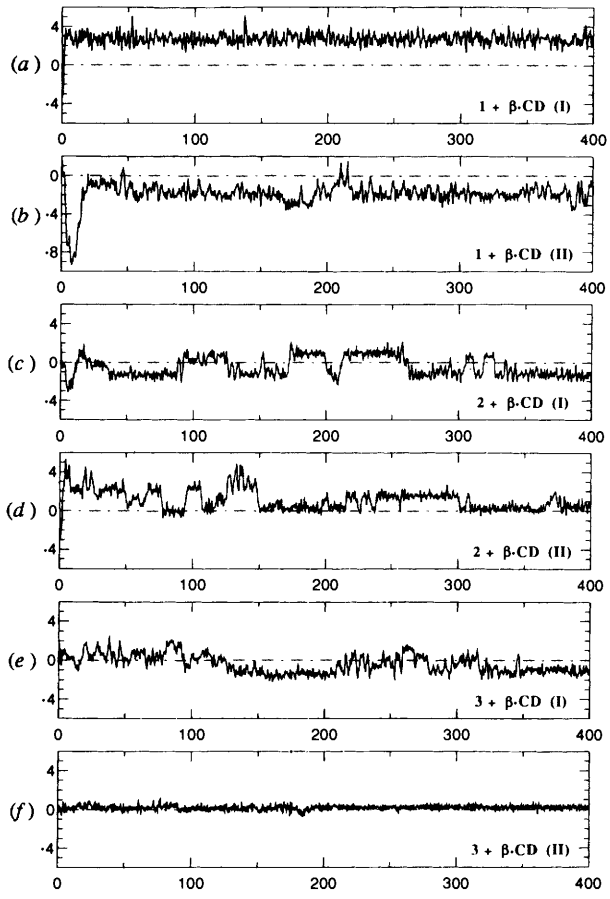


Fig. 2 Selected time evolution traces from MD simulations *in vacuo* for distances between the mass centre of β-CD and the centre of its closest guest ring, showing the relative host–guest movements in complexes of **1**, **2** and **3**. The traces refer to the ring C of **1** orientated as (*a*) **I** and (*b*) **II**; the ring A of **2** orientated as (*c*) **I** and (*d*) **II**; the phenylmethyl group of **3** orientated as (*e*) **I** and (*f*) **II**. In the ordinate and abscissa are quoted the distance (in Å) of the ring centre from the assumed origin (Fig. 1) of the β-CD symmetry axis and the evolution time (in ps), respectively.

Molecular dynamics

The results of the MD simulations of the β-CD complexes with **1**, **2** and **3**§ are presented in terms of time evolution of the distances, along the symmetry axis of the β-CD, between the mass centre of β-CD and the centre of the closest ring of the guest. The assumed positive direction of the axis is denoted by the dashed arrow in Fig. 1.

In the complexes of **1** at the equilibrium, the cyclohexenyl ring (C) is the guest fragment closest to the host and fluctuates at distances of 3 and 2 Å from the cavity centre, in the orientations **I** and **II**, respectively [Fig. 2(*a*) and (*b*)]. During the simulation time, the type-**II** complex, more markedly than type-**I**, attains a structure in which a portion of the guests C ring is still anchored to the β-CD rim. In both complexes it cannot therefore be decided whether the internal cavity protons of the host are sufficiently shielded for NMR evidence of inclusion.

§ The β-CD was chosen as common reference host for **1**, **2** and **3** since a previous study[8] performed for guest **3** showed a higher degree of penetration of **3** into β-CD than in α-CD (partial inclusion) and γ-CD (non-inclusion).

Table 1 Results of MD simulations *in vacuo* of β-CD, compounds **1**, **2** and **3** alone, and in the complexed state. The average values of total $\langle E_T \rangle$, bond $\langle E_b \rangle$, van der Waals $\langle E_{VDW} \rangle$ and electrostatic $\langle E_{ELEC} \rangle$ energies (kcal mol^{-1})

Compound	Orientation	$\langle E_T \rangle$/kcal mol^{-1}	$\langle E_b \rangle$/kcal mol^{-1}	$\langle E_{VDW} \rangle$/kcal mol^{-1}	$\langle E_{ELEC} \rangle$/kcal mol^{-1}
β-CD		134.93	3.33	−18.54	−94.17
1		18.85	5.46	2.32	−8.41
2		12.64	5.67	3.73	−17.66
3		24.41	0.68	1.11	−6.96
1 + β-CD	**I**	135.73	33.10	−31.35	−130.24
	II	153.94	33.36	−33.51	−113.80
2 + β-CD	**I**	144.85	33.73	−34.42	−124.53
	II	129.81	33.64	−34.11	−138.46
3 + β-CD	**I**	130.19	4.47	−44.77	−105.48
	II	114.78	4.03	−46.25	−116.24

This is not the case for complexes of **2**, whose attained equilibria states are close to the starting minimized structures. The guest fluctuates about these positions with a maximum deviation of ±2 Å, in the timescales of 100 and 50 ps, in the **I**- and **II**-type complexes, respectively [Fig. 2(*c*) and (*d*)]. Assuming the mass centre of β-CD is nearly equidistant (*ca.* 3.5 Å) from the rims, it follows that both A and B rings of **2** reside inside the macrocycle during the evolution time. This MD result is in contrast (at least on the timescale studied) to the NMR experimental evidence of non-inclusion of **2**.

In accord with experiment,[8] molecule **3**, during the entire simulation time, independent of the relative orientation with respect to β-CD, remained firmly entrapped within the torus with very small fluctuations about the equilibrium position [Fig. 2(*e*) and (*f*)].

Energetics of inclusion are shown in Table 1. A general decrease in the average energies of uncomplexed host and guest was attained after simulating the host–guest complex. Most noteworthy is the significant decrease in the total potential energy attained by the complex of **3** whose effective formation has been demonstrated.[8] The complexes of **1** and **2** are less favoured on energetic grounds with respect to the complexes of **3**. Interestingly, in the case of **1** and **2**, significant lowering of electrostatic energies is observed to counterbalance the corresponding high increase in the total bond energies after docking. This denotes forced bond deformations caused by accommodation of these guests inside the cavity.

The above simulations were of complexes *in vacuo*. A more realistic and computationally more exacting model, in which the solvent is explicitly considered, seemed therefore in order (*i*) to check the possibility of attainment of MD results consistent with experiments for all the considered systems and (*ii*) to ascertain the medium effects on the dynamics of embedded molecules.

The results of MD simulations with explicit solvent, in terms of time evolution of distances as defined above for simulations *in vacuo*, are illustrated by selected traces in Fig. 3. From these it may be easily inferred that in complexes **1** and **2** the distances of the host–guest mass centres evolve regularly from the start (t = 0 ps), without significant excursions forward and backward, until attainment of an equilibrium value geometrically corresponding to definitive guest extrusion. The guests do not go on beyond the mouth of the host because of border effects on the outer fragment of the extruded molecule due to the limited size of the water sphere used. As an example, Fig. 4 shows the last step of the simulation run of the complex of **1** with β-CD oriented as **I**.

This clearly was not the case for complex **3**, whose trajectories are markedly different from those of **1** and **2** and thus correspond to permanent residence of **3** inside β-CD.

These MD results consistently reproduce the NMR evidence of **1** and **2** non-inclusion, and **3** inclusion, into β-CD. In general, this implies that for charged guests the explicit water in MD simulations is a factor making more realistic and sound the model, and that, accordingly, the water molecules play an important role in determining the effective inclusion or non-inclusion of a given drug.

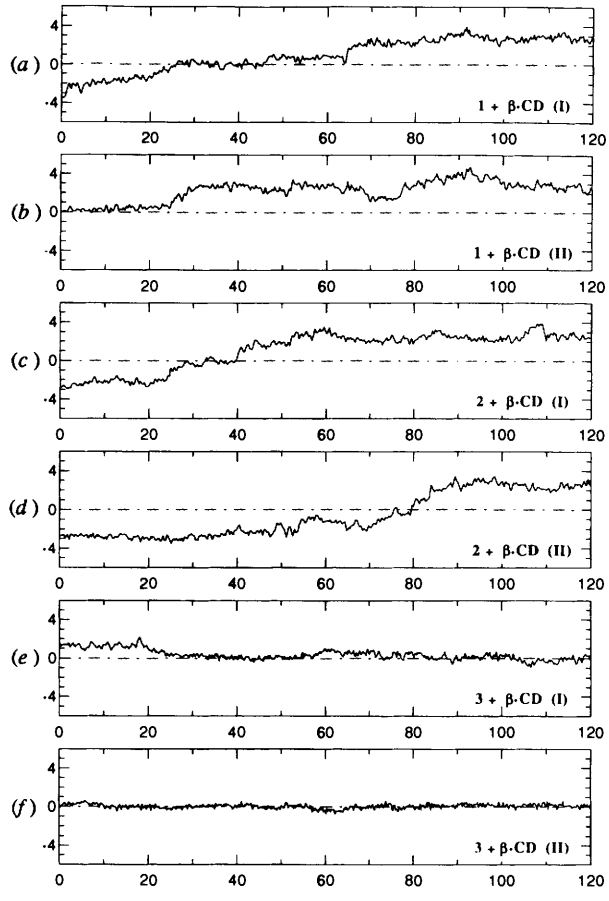


Fig. 3 Selected time evolution traces from MD simulations with the water sphere, for distances between the mass centre of β-CD and the centre of its closest guest ring, showing the relative host–guest movements in complexes of **1**, **2** and **3**. The traces refer to (*a*) the ring C of **1** orientated as **I**, (*b*) to the ring A of **1** orientated as **II**, (*c*) the ring C of **2** orientated as **I**, (*d*) the ring A of **2** orientated as **II**, (*e*) the phenylmethyl group of **3** orientated as **I**, (*f*) and **II**. In the ordinate and abscissa are quoted the distance (in Å) of the ring centre from the assumed origin (Fig. 1) of the β-CD symmetry axis and the evolution time (in ps), respectively.

By assuming that two main competitive equilibria operate, *i.e.* solvation of the guest and complexation of the unsolvated guest by β-CD, it follows that the relative values of their equilibrium constants may affect the inclusion process. To have an idea on the relative strength of the guest–water and guest–host complexes we simulated each solvated guest in order to evaluate the water distribution around the molecules, their affinity with water and related energetics.

In Table 2 are summarized the results of MD simulations of **1**, **2** and **3** within the water sphere. All the energies were

Table 2 Summary of MD simulation results for compounds **1**, **2** and **3** in the water sphere: average number of water molecules ($\langle N_W \rangle$) and number of water molecules per single atom of the compound ($\langle N_W \rangle / N_A$), in a shell 5 Å thick; average total energies (kcal mol^{-1}) in the shell (5 Å) of each compound relative to water molecules ($\langle E_T \rangle_{W-W}$) interactions, and to water molecules with the compound ($\langle E_T \rangle_{W-C}$) interactions; calculated average total non-bonded interaction energies (kcal mol^{-1}) of an atom of the compound with a water molecule ($\langle E_T \rangle_{W-C} / \langle N_W \rangle N_A$) and of a water molecule with a water molecule. N_A is the number of heavy atoms of each compound

Compound	N_A	$\langle N_W \rangle$	$\langle N_W \rangle / N_A$	$\langle E_T \rangle_{W-C}$/ kcal mol^{-1}	$\langle E_T \rangle_{W-W}$/ kcal mol^{-1}	$(\langle E_T \rangle_{W-C} / \langle N_W \rangle N_A)$/ kcal mol^{-1}	$(\langle E_T \rangle_{W-W} / \langle N_W \rangle^2)$/ kcal mol^{-1}
1	15	45.54	3.04	−71.82	−280.75	−0.105	−0.135
2	16	47.46	2.97	−72.81	−304.47	−0.096	−0.135
3	23	59.30	2.58	−84.32	−373.17	−0.062	−0.106

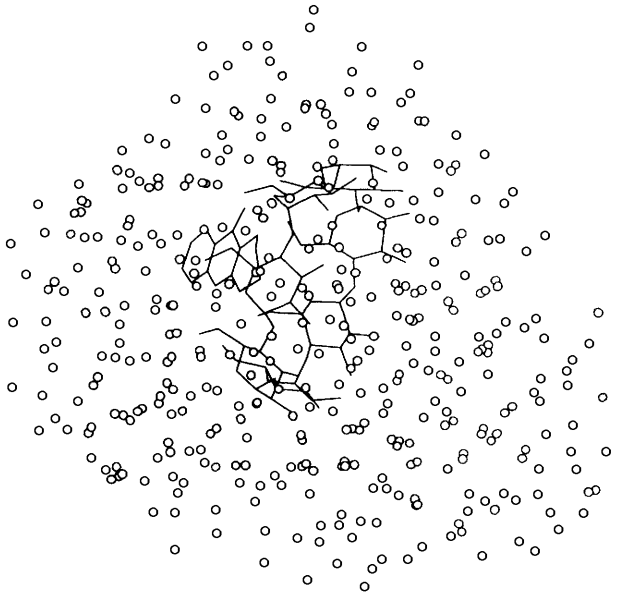


Fig. 4 Structure sampled at t = 120 ps of the simulation run in the water sphere of **1** + β-CD orientated as **I**. For clarity, all hydrogen atoms are deleted and the circles represent the oxygen of the water molecules.

obtained for a number of water molecules N_W (the meaning of the other symbols is defined in Table 2) that are situated within a shell 5 Å thick. Significant data were provided by (*i*) the average density factor ($\langle N_W \rangle / N_A$), which indicates the mean number of water molecules per heavy atom of the compound, (*ii*) the average interaction energy per heavy atom of the compound and per water molecule ($\langle E_T \rangle_{WC} / \langle N_W \rangle N_A$) and (*iii*) the average interaction energy per water molecule with the other water molecules ($\langle E_T \rangle_{WW} / \langle N_W \rangle^2$). All these indicate that compound **3** is the less hydrophilic one with respect to the parent compounds **1** and **2**. This is reasonably attributable to the phenylmethyl group that makes molecule **3** able to more easily lose water molecules and to make free a portion of its hydrophobic surface for the lipophilic process of inclusion. The density factor shows that for the compounds **1** and **2** a greater number of water molecules than for **3** must be removed for this to happen. Further, the cohesion energy of each water molecule within the shell for each atom of the compound, as well as for every other water molecule, is lower for **3** than for molecules **1** and **2**.

By considering that the three compounds have similar 1,2,3,4-tetrahydro-9-aminoacridine skeletons, the smaller water affinity of **3** may be attributed to the contribution of the phenylmethyl group. Interestingly this is the molecular portion that is anchored inside the cavity when **3** is complexed.

In the case of guests **1** and **2**, it should also be considered that the MD simulations with explicit water work well to pull out the guests not only for the greater affinity of the solvent towards the cation, but also for making more significant the contributing stress effect of the increased internal energy terms in the complex (Table 1).

The results attained in this work clearly indicate that an oversimplified model, such as that of assembling the complex *in vacuo* and using a continuum-like solvent treatment, may lead to incorrect scientific conclusions. In water, the hydrophilic–lipophilic groups on a series of related candidate guests may feature these molecules as a function of their relative interactions with the medium. Such interactions, expecially for charged species cannot be modelled in the absence of explicit water molecules or at least as we have done by using a distance dependent relative permittivity that is often used to mimic solvent. This clearly indicates that, in the absence of safe experimental data, the solvation effects must be taken into account when predicting effective inclusion or non-inclusion of a guest into CDs exclusively on the basis of a theoretical model.

Simulations with explicit water and periodic conditions, in which border effects are avoided, could provide further information concerning the guest–water molecules interactions which may influence the complexation equilibria. This task, that could also highlight unanswered general questions concerning inclusion and non-inclusion of cations and anions, is an argument of a separate study we have initiated.

Acknowledgements

Work was supported by MURST of Italy. Thanks are also due to Warner-Lambert and Hoechst-Russell that provided the samples of **1** and of **2** and **3**, respectively.

References

1 D. Duchêne, *New Trends in Cyclodextrins and Derivatives*, ed. de Santé, Paris, 1991.
2 J. Szejtli, *Cyclodextrin Technology*, Kluwer, London, 1988.
3 R. Breslow, in *Inclusion Compounds*, eds. J. L. Atwood, J. E. D. Davies and D. D. MacNicol, Academic Press, London, 1984, vol. 3, pp. 473–508.
4 P. Tallal, *J. Clin. Psychopharmacol.*, 1985, **5**, 272.
5 *Drug Fut.*, 1990, **15**, 1152 and references cited therein.
6 *Drug Fut.*, 1989, **14**, 643 and references cited therein.
7 G. M. Shutske, F. A. Pierrot, K. J. Kapples, M. L. Cornfeldt, M. R. Srewczak, F. P. Huger, G. M. Bores, V. Haroutunion and K. L. Davis, *J. Med. Chem.*, 1989, **32**, 1805.
8 M. E. Amato, G. C. Pappalardo and B. Perly, *Magn. Res. Chem.*, 1993, **31**, 455.
9 P. Job, *Ann. Chim.*, 1928, **9**, 113.
10 B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan and M. Karplus, *J. Comput. Chem.*, 1983, **4**, 187.
11 B. H. Besler, K. M. Merz, Jr. and P. A. Kollman, *J. Comput. Chem.*, 1990, **11**, 431.
12 J. J. P. Stewart, MOPAC-6, QCPE 455, 1990.
13 G. Bandoli, A. Dolmella, S. Gatto and M. Nicolini, *J. Chem. Crystallogr.*, 1994, **24**, 301.
14 K. Harata, *Bull. Chem. Soc. Jpn.*, 1988, **61**, 1939, and references cited therein.
15 R. J. Bergeron in *Inclusion Compounds*, eds. J. L. Atwood, J. E. D. Davies and D. D. MacNicol, Academic Press, London, 1984, vol. 3, pp. 391–443.

Paper 5/05168A
Received 2nd *August* 1995
Accepted 9th *October* 1995