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Conformational and circular dichroism studies on cyclodextrin inclusion complexes

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Abstract: A framework aimed at elucidating the structure of inclusion complexes between cyclodextrins (CDx) and several chromophores of photochemical interest is presented. The scheme is composed by a set of Molecular Mechanics calculations, Monte Carlo simulations including solvent effects and reproduction of the Induced Circular Dichroism (ICD) using quantum mechanically calculated properties combined to the Kirkwood -Tinoco expressions for the induced rotational strength . Examples of molecular recognition between α,β and γ -CDx and a variety of chromophores of fundamental as well as of applicative interest , such as phenols, dimethoxybenzenes and buckminsterfullerene are examined. The method proposed proves to be a suitable instrument to elucidate different geometrical configurations and to be able to gain insight into the relationship between structural and dynamic properties of the complexes studied.

Introduction

Supramolecular self-assembly has received recently a great deal of interest in several fields of Chemistry and Biology, as it provides the physical basis for molecular recognition , formation of enzyme-complexes with substrates and is also related to protein folding. A remarkable model for this phenomenon is represented by the inclusion of a variety of organic compounds of molecules of suitable size in cyclodextrins (CDx), cyclic oligosaccharides consisting of six (α-CDx) , seven (β-CDx) or eight (γ-CDx) D(+) glucopyranose units linked by α-(1,4) bonds and containing a central, hydrophobic cavity with a diameter of 5-8 Å (1). The stability of the inclusion is determined by the fit of the molecular shape of the guest to the surface of the cavity, with intervention of van der Waals forces, hydrogen bonding, decrease of strain energy and release of high energy water molecules from the cavity.The high solubility in water of the inclusion complexes of hydrophobic molecules in CDx has been exploited for a number of applications in pharmaceutical chemistry , food technology and plant protection industries. Moreover, due to their capability in binding substrates quickly, selectively and reversibly and to behave as catalysts in many chemical reactions, CDx can be considered good model enzymes. It is , therefore, of fundamental importance to understand the physics of complexation, i.e. the driving forces which govern the association as well as the geometrical and thermodynamic features of the most stable structures. Spectroscopic investigation of these complexes presents two main advantages , i.e. the lack of interference of the bands of the macrocycle in the far U.V. with those of most chromophores, and the induction of chirality in the guest molecule, with possibility of detecting this new property by means of Circular Dichroism techniques. On the other hand , photophysical and photochemical studies provide a wealth of information on the dynamics of deactivation of the excited states of the included molecules , sometimes dramatically different from that of the free molecules (2). It would be therefore highly desirable to dispose of a tool that permits a rapid connection between the structure and the spectroscopic and photophysical properties of these supermolecules. This tool was found in a combination of different theoretical techniques, such as Molecular Mechanics calculations, Monte Carlo simulations including solvent effects and the reproduction of the Induced Circular Dichroism . This latter provides a reliable test of the most stable structures previously calculated due to its sensitivity to the geometric set-up of the complex.
In this communication we review the results of the studies carried out recently in our Institutes on the inclusion complexes of CDx and some organic compounds of photochemical interest like phenols, methoxybenzenes and fulllerene C_{60}.

2. Theoretical methods

Starting from geometries of guest and host previously optimized using the Allinger's force field (3) , in the first step we search for coarse local minima along a complexation pathway obtained from stepwise transfer of the guest into the CDx cavity. The second type of calculation consists in a Dynamic Monte Carlo (DMC) simulation with 1000 steps per run at a constant temperature of 300 K. The conformational space is explored by varying stochastically i) the dihedral angles between adjacent glucose subunits of CDx, ii) the host-guest distance and iii) the relative orientation of host and guest. Each generated complex geometry is fully minimized within the MM3-92 force field (4). Particularly important is, in this step, the inclusion of the solvent effect, simulated by a continuum model using the MSEED program (5), which allows an estimate of the solvent accessible molecular surface and the solvation free energy given by hydrophobic and hydrophylic terms. In this model a stochastically generated configuration is accepted when the following modified Metropolis criterion is satisfied :

$$P(\Delta E, \Delta G_{solv}) = e^{-\frac{\Delta E}{k_B T}} e^{-\frac{w\sum_{i=1}^{n} \Delta S_i \sigma_i}{k_B T}} \qquad (1)$$

where P represents the probability of acceptance of a conformation with potential energy ΔE and solvation energy ΔG_{solv} between the actual and the last accepted structure , ΔS_i and σ_i give the solvent accessible area and the atomic solvation parameter respectively for the i-th atom . The parameter w weights the hydrophobic (w> 0) or hydrophylic (w<0) contributions to the solvation energy.
The third step of calculation consists in the reproduction of the Induced Circular Dichroism for the generated energy minima. Out of the three mechanisms that generally describe the induction of rotatory power in a complex, i.e. the one electron, the dipole-dipole and the magnetic-electric interaction, the second appears the most important in this case. A particularly useful approximation is obtained introducing in the Kirkwood's equations the polarizability of the bonds of the chiral macrocycle in place of the original dipole-dipole interaction scheme(6). Along this approximation, the pertinent expression of the rotatory strength for a transition $0 \rightarrow a$ is given by :

$$R_{0a} = \pi \nu_a \mu^2_{0a} \sum_j \frac{\nu_{0j}^2 (\alpha_{33} - \alpha_{11})_j (GF)_j}{c(\nu_{0j}^2 - \nu^2_a)} \qquad (2)$$

$$(GF)_j = \frac{1}{r_j^3}\left[\mathbf{e}_{0a}\mathbf{e}_j - \frac{3(\mathbf{e}_{0a}\mathbf{r}_j)(\mathbf{e}_j\mathbf{r}_j)}{r_j^2} \right] \mathbf{e}_{0a} \times \mathbf{e}_j \mathbf{r}_j \qquad (3)$$

where $\mathbf{e}_{0a}$ and $\mathbf{e}_j$ are unit vectors along the electric transition moment μ_{0a} and parallel to the j-th bond respectively ; ν_{0j} and ν_a are frequencies of the electric transitions of host and guest, that are located at a distance $\mathbf{r}_j$, and α_{11},α_{33} represent bond polarizabilities at zero frequency, parallel and perpendicular to the symmetry axis of the bond j-th. In eq.(2) the energies and electric moments can be calculated by quantum mechanical methods , as in the present case (CNDO/S), or extracted from electronic spectra. Being the rotational strength directly proportional to the factor expressed by eq. (3), it turns out that the method is quite sensitive also to slight variations of the geometric set-up.

3 Results

1:1 Complexes of Phenols and β-CDx

The inclusion of phenols in CDx is particularly interesting as it provides a suitable model for the interaction of aromatic amino acidic residues within protein matrices. This is due to the fact that, beyond non specific van der Waals and hydrophobic forces , phenols can form hydrogen bonds with the OH

groups of the CDx macrocycle. We examined a series of derivatives with increasing degree of methylation, going from phenol itself to p-cresol, 2,4 and 3,5 dimethylphenol (DMP) and 2,4,6 and 3,4,5-trimethylphenols (TMP) included in β-Cdx (7). A complexation pathway diagram calculated for phenol and 2,4,6-TMP is shown in fig.1.

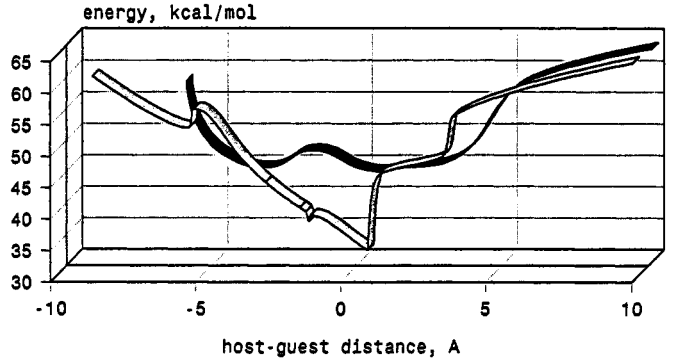

Fig. 1 Complexation coordinate of Phenol (light) and 2,4,6-TMP with β-CDx. Potential energy evaluated by MM2-87 force field.

A further exploration of the minima with the Dynamic Monte Carlo method without inclusion of solvent in the model showed that the complex of phenol-β-CDx is highly flexible , with a number of comparable minima spread over the conformational space. The insertion of bulkier chromophores restricts the flexibility of the system , giving rise to tighter and better defined complexes: for example, the mean change in atomic coordinates, calculated for two consecutive conformations of phenol and p-cresol during a Monte Carlo simulation run in the region of stable energy potential minima, is 0.88 Å and 0.38 Å, respectively, and decreases further for the TMP's. Depending on the minimum intermolecular distances and on the corresponding potential energy, the inclusion complexes of the methylated phenols can be classified in three groups : phenol and p-cresol are fully imbedded in the cavity ; the DMP's, with the exception of 2,6 DMP , present a broad range of equivalent minima corresponding to the guest located slightly off the center of the cavity , at a typical host-guest distance < 2 Å ; the TMP's and the bulkiest of the DMP present minima well separated by relatively high energy barriers , with the guest located near the rims of the cavity. This classification broadly reflects the different van der Waals volumes of the guests, showing that, say, the large steric hindrance of three neighboring methyl groups prevents the TMP from penetrating the cavity. However , due to the large number of possible potential energy minima still found for selected geometric arrangements (e.g. 148 and 78 non identical structures for phenol and 2,4,6-TMP respectively in a range of 20 Kcal/mole), it was necessary to introduce some more constraints to the model, like the cominimization of the conformational and solvation energies during the Monte Carlo annealing (8). This implementation gives rise to a tighter structure: in the case of phenol, when the hydrophobic term is introduced , the accessible surface in correspondence of the initial local minimum decreases from 710 $Å^2$ to 540 $Å^2$. In the first case the phenol molecule is able to escape from the cavity as soon as a stable conformation is reached, whereas in the second the guest remains imbedded in the CDx cavity. A convincing test of the reliability of the geometries obtained and of the effectiveness of the inclusion of solvent in the model is given by the response of the calculated ICD. This spectrum shows two bands of opposite sign , the first negative , in the region of the lowest transition ($^1B_{2u}$ pseudosymmetry, λ = 270 nm) and the second , positive, in corrispondence of the second singlet state ($^1 B_{1u}$, λ= 220 nm). This pattern could be, in principle, predicted by a simple model previously proposed, showing that in case of axial inclusion of phenol in a truncated cone-shaped host, a positive signal should be detected for electronic transitions polarized along the central axis of CDx and a negative one for those normal to it (9). The reversal should hold for chromophores inserted equatorially . However this pattern can occur for a molecule placed outside the cavity (10).The present work shows that some macrocycles , like β-CDx are highly nonsymmetrical in solution, with a geometry deviating considerably from a circular structure and with a tilt angle between adjacent, not identical, glycose units. This result bears important consequences in the calculation of the optical activity of these complexes, as the asymmetric carbons of these units represent the main centers of chirality of the supermolecule. Different results were found by minimizing the potential energy alone , and by adding the hydrophobic solvation energy and the total solvation energy. Out of the 100 structures of lowest energy for phenol, it was found that while the first and third variant gave comparable results (14 and 36 structures accepted on the

basis of the sign and intensity ratio between the two ICD bands, and guest to host distances with similar values), a remarkable improvement (100 structures accepted) is observed with the second variant and the factor w sufficiently large. Fig. 2 shows the geometry of two probable complexes obtained with this method for phenol and 2,4,6-TMP.

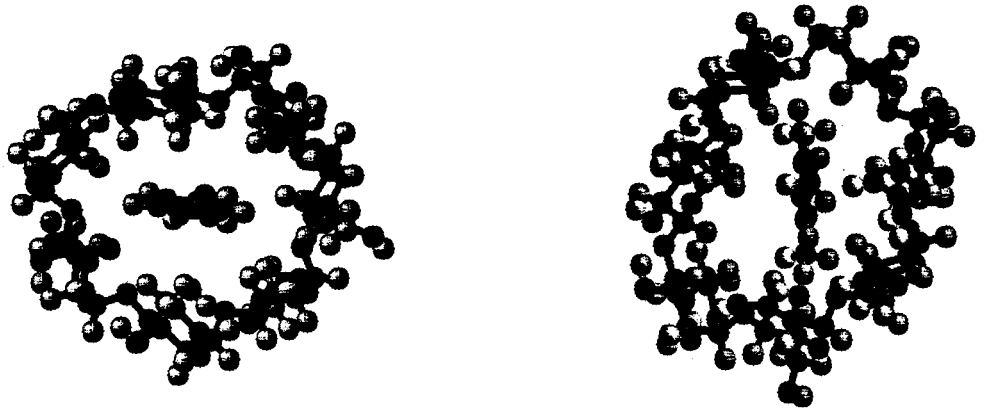

Fig.2 Low energy geometry structures (MM3 force field) calculated for phenol (left) and 2,4,6-TMP(right) , including solvation effects.

While phenol is completely imbedded in the cavity, the bulkier 2,4,6-TMP is attached to one side of the cavity , with distortion of the macrocycle; in both cases, however , several hydrogen bonds between adjacent OH groups are formed , a feature which renders more rigid the cone shaped structure of β-CDx and which was confirmed by crystallographic data. As a general result , it was found that solvation reduces the multidimensional conformational space of phenol complexes to a smaller subspace, and in the case of the larger methylated compounds , to a limited number of well defined structures. This fact is reflected in a general sharpening of the electronic spectra and in the detection of a single fluorescence lifetime which would be hardly consistent with population of many minima in these complexes (11).

Higher order complexes of chromophores with CDx.

The ability of several chromophores to form higher order complexes, with at least two or more macrocycles and possibility of generating nanotubes is another important aspect of macromolecular self-assembly. The investigation of complexes of 1,4 and 1,2 Dimethoxybenzenes with two α-CDx allowed a direct connection between the structural properties and the photophysical and photochemical behavior of these compounds (12). In fact, the spectroscopic and fluorescence properties of these inclusion complexes point to a less polar environment of the guest with extrusion of water molecules from the cavity. This phenomenon appears particularly effective in the case of 1,4-DMB in α-CDx ; the analysis of the triplet decay and of its temperature dependence at high CDx concentrations is consistent with a model

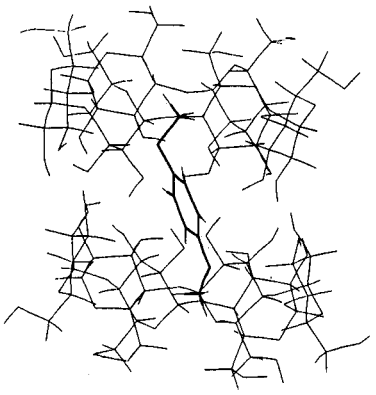

Fig.3.Low energy complex of 1,4 DMB in 2 α-CDx (Potential energy= 74.3 kcal/mole, solvation energy hydrophobic surfaces = 10.4 Kcal/mole)

predicting the guest molecule fully included in the cavity formed by two cyclodextrin molecules with axial geometry (fig.3). On the other hand, the complexes of the bulkier 1,2-DMB are calculated with a more undefined geometry, with several equivalent minima and unreduced degrees of freedom of host and guest, a feature that is in line with a mean disappearing ICD spectrum in the region of S_1.

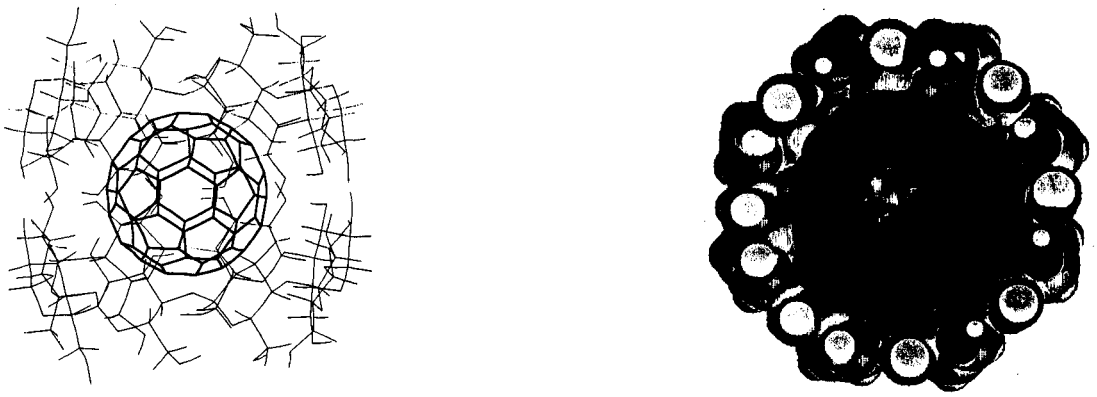

Fig.4 Two different views of a low energy complex of C_{60} with two γ-CDx molecules.

Also the very large fullerene C_{60} (diameter 7 Å) can be included in cyclodextrins : recently a stable complex of this fullerene with γ-CDx was sinthetized and the NMR analysis showed a highly symmetric , bicapped structure of 1:2 stoichiometry (13). Our results are consistent with this physical picture, being able to reproduce the main features of the ICD spectrum of this complex in correspondence of a tight bonded , low energy calculated geometry (fig.4) (14). Interestingly, the largest positive band , calculated at 250 nm and attributed to the second allowed $^1T_{1u}$ singlet of C_{60} , is reproduced with the right sign only for a few, very tight and low energy geometries of the complex,while for looser or less symmetric arrrangements the sign is calculated negative. Therefore we concluded that this band can be assumed as diagnostic of the effective host-guest recognition for this large complex.
Moreover, due to the distortion of the included carbon cluster, with subsequent decrease of symmetry , a rotational strength is detected also for states strictly forbidden in case of I_h , unperturbed symmetry. As already pointed out in case of symmetry lowering for fulleroprolines (15), the possibility of detecting hidden states of fullerenes represent another advantage deriving from the ICD investigation of these supermolecules.

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