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ORIGINAL ARTICLE

Circular dichroism and theoretical studies on the inclusion of the antimalarian drug Licochalcone-A in β-cyclodextrin

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Abstract The Induced Circular Dichroism of the complex formed by inclusion of Licochalcone-A (a powerful antimalarian of natural origin) and β-cyclodextrin was measured and calculated. The association constant was determined by titration experiments of ICD and fluorescence. The lowest energy conformations of the complex were obtained through docking procedures and their Circular Dichroism was evaluated within the Tinoco framework. The geometry of the complex was found to be consistent with the main experimental features.

Keywords Licochalcone · Malaria · Circular Dichroism

Introduction

The efforts to find antimalarial compounds of natural origin with possibly large availability, easy vehiculation in the human body and high effectiveness against resistant strains of the *Plasmodium falciparum*, prompted us to study the molecule of Licochalcone-A, a natural component of Licorice extracted from at least three species of *Glycyrrhiza*, forming the chinese drug Gan Cao [1]. Chalcones are structurally simple flavonoids present in many plants with a wide spectrum of pharmacological properties, ranging from antibacterial to antifungal and immunosuppressive activity [2]. Licochalcone-A, in particular, has been shown to be effective in inhibiting in vitro growth of the human malaria parasite [3] and to exert a potent activity against human pathogenic protozoan species of *Leishmania* [4]. This molecule is insoluble in water and the problem of its vehiculation in the human body can be overcome either by suitable substitution with more hydrophilic groups or, more simply and without the risk of altering the original properties of the drug, by including it in highly soluble macrocycles such as the cyclodextrins. Beyond the usefulness of an increased bio-availability of the compound, this kind of complexation lends itself as a model for the inclusion of the drug in more realistic biological systems such as the lipophilic pouches of suitable proteins.

In this work we have dealt with the characterization of the complex Licochalcone-A:β-cyclodextrin through measurement of its Induced Circular Dichroism (ICD) and fluorescence and calculations based on Molecular Mechanics and Semi-empirical quantum chemistry, aimed at gaining insight into the most favorable energetic setups of the supermolecule. It was found that only few, energetically favorable, arrangements of the host-guest give rise to the right sign of the sequence of the ICD bands. Moreover the geometry found for the complex appears to be compatible with the value of the complexation constant derived both from ICD and fluorescence measurements.

Experimental

Licochalcone-A (**1**), from Calbiochem and β-cyclodextrin from Serva were used as received. Water was purified by passage through a Millipore MilliQ system. All the solvents used were of spectroscopic grade.

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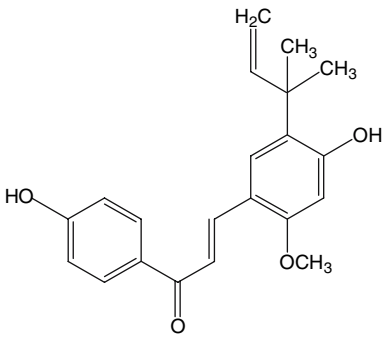
Ultraviolet absorption spectra were recorded on a Perkin-Elmer λ45 spectrophotometer. Due to the very low solubility of the drug in water the aqueous solutions were prepared with 16% ethanol. Circular Dichroism spectra were obtained with a Jasco J-715 spectropolarimeter. To prepare the samples containing cyclodextrin, aliquots of an ethanolic solution of **1** were introduced in flasks, the solvent was evaporated and suitable volumes of aqueous cyclodextrin solution were added. Fluorescence emission spectra were obtained on a LS50B (Perkin Elmer) spectrofluorimeter. Lifetimes were determined by means of a conventional time-correlated single photon counting system (IBH Consultants Ltd.) in air-equilibrated solutions, with excitation wavelength of 373 nm. The absorbance of the solutions was ca. 0.3 in the usual 1 cm cell. The instrumental response function had a full width at half maximum of 1.4 ns and the resolution limit with deconvolution was ca 0.15 ns.

The assessment of the best complexation model and the determination of the association constant of the complex were performed by global analysis of spectra (ICD or fluorescence) obtained at 5–6 different cyclodextrin concentrations, using the whole wavelength range for the calculations (commercially available SPECFIT/32 program from Spectrum Software Associates). The temperature of all the experiments was 295 K.

Results and discussion

Licochalcone-A is a flavonoid compound of the chalcones family, formed by α,β-unsaturated bisphenylic ketones. In Licocalchone-A the chalcone skeleton is substituted with two phenolic hydroxyl groups, one methoxy group and an isoprenoid side chain (Scheme 1). The α,β-unsaturated ketone group is thought to be able to alkylate *N*-acetyl-L-cysteine and other biomolecules having thiolo groups, while the phenolic hydroxy groups might behave as free oxygen-radical scavengers.



Scheme 1 Licochalcone-A, **1**

The absorption spectrum of **1** in several solvents is reported in Fig. 1. In aqueous solution (with 16% ethanol) it shows an intense band peaked at 383 nm, shifting to 362 nm in CH_2Cl_2 and to 373 nm in cyclohexane; a series of bands of lower intensity at ~310, 260, 235 nm are less dependent on solvent. The presence of 10^{-2} M β-cyclodextrin shifts to the blue by ca. 5 nm the lowest energy band (maximum at 378 nm with respect to 383 nm in the aqueous medium) and generally decreases the intensity of the absorption. The fluorescence spectrum consists of a large band with maximum around 500 nm, solvent dependent. The emission quantum yield in aqueous medium is ca. 6×10^{-3} and the lifetime is $\leq$0.15 ns, the resolution limit of our instrumentation.

The ICD spectrum of **1** in presence of β-cyclodextrin is characterized by weak bands with positive sign at 260, 306 and 355 nm and a very weak negative band centered at 400 nm Titration by β-cyclodextrin in phosphate buffer 10^{-2} M pH 7.4 allowed a 1:1 binding stoichiometry and Log (K_{ass}/M^{-1}) = 2.5 ± 0.17 to be assessed. Titration experiments with fluorescence detection led to a value of Log (K_{ass}/M^{-1}) = 1.7 ± 0.14 M^{-1}. The absolute ICD spectrum of the complex is represented in Fig. 2. Docking of Licochalcone-A in β-cyclodextrin was simulated using the module Discover of the package InsightII [5]. Four initial reciprocal dispositions of the host and guest molecules were imposed, i.e. with **1** facing the large (secondary) rim with the isoprenoid chain (a), and with the phenolic group (b) respectively, and the same geometries

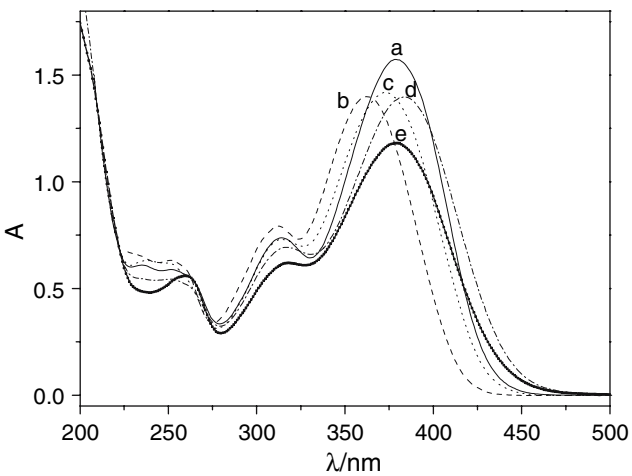


Fig. 1 Absorption spectra of 5.82×10^{-2} M **1** in ethanol (a), dichloromethane (b), cyclohexane (c), water (16% ethanol) (d), 10^{-2} M β-cyclodextrin (e). Cell 1 cm, T = 295 K

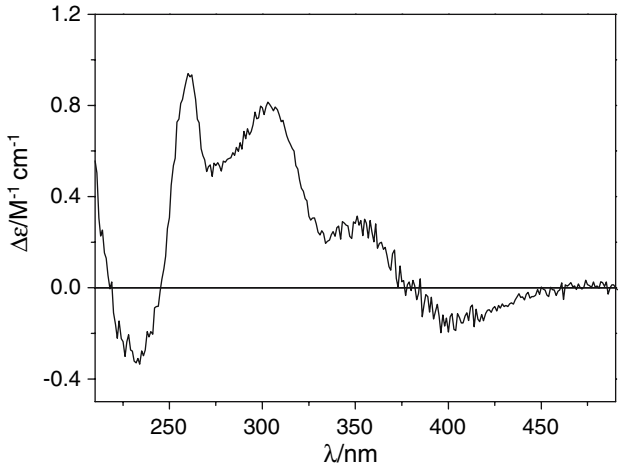


Fig. 2 ICD of the 1:1 β-cyclodextrin:**1** complex in phosphate buffer 0.01 M, pH 7.4

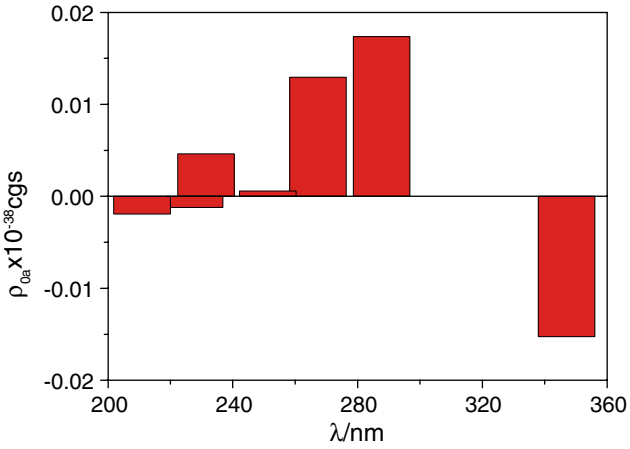


Fig. 4 Calculated ICD for the 1:1 β-cyclodextrin:**1** complex

of **1** facing the small entrance of the cavity (primary rim) (c and d, respectively). Starting from an energy of 201.89 and 93.52 kcal/mol for the host and guest respectively, the docking procedure allowed minimum energy setups of 261.10, 261.66, 272.98 and 265.43 kcal/mol for the four geometries initially outlined. Among the docked structures (Fig. 3), (a) shows the best inclusion with the isoprenoid group and part of the methoxy aryl substituent inserted in the cavity, leaving the ketone, the vinylic and the phenolic groups outside and faced to the secondary rim. All the other three configurations show just a superficial attachment of the guest to the secondary (b) and primary (c and d) rims. These findings are consistent with the modest value of the association constant and the low ICD experimentally determined. The optimized geometries were taken as a starting point for the calculation of the ICD, carried out according to the formulation of Kirkwood–Tinoco, which replaces the original dipole-dipole interaction scheme in the Kirkwood's equations by the polarizbility of the bonds of the chiral macrocycle [6]. This method has been successfully employed in the past to elucidate the structure of a number of host-guest complexes [7–9]. In this work the energies and the transition electric dipole moments were calculated using the CNDO/S method, with the Mataga–Nishimoto parametrization for Coulomb integrals. The calculated energies predict a sequence of bands at 347 (very weak in absorption), 288 (corresponding to the HOMO → LUMO promotion), 267, 251 (strong), 231, 228, and 210 nm, in reasonable agreement with the main experimental features as far the ordering is concerned, whereas the energies are rather overestimated, especially for the lower bands.

The calculated ICD in correspondence of the minima found by docking, (Fig. 4), shows a satisfactory agreement for the sequence of signs and intensities just for the (a) configuration, whereas all the other geometries give a completely wrong sequence of signs (all negative). Therefore we conclude that the (a) configuration corresponding to the lowest energy and to a deeper inclusion in the host cavity, is the most probable. The rather peripheral geometric set up, and the consequent low association constant can play an important role of the drug release rate once it reaches the crucial protein sites.

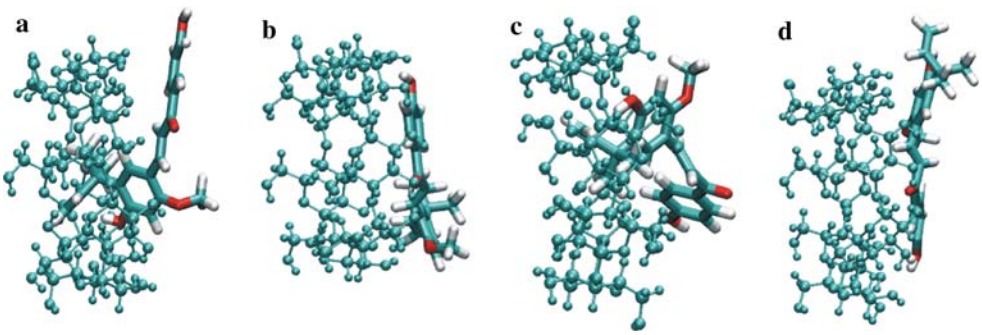


Fig. 3 Lowest energy configurations calculated for setups a, b, c and d (see text)

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