Journal of Photochemistry and Photobiology A: Chemistry 266 (2013) 12–21

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Spectroscopy and dynamics of topotecan anti-cancer drug comprised within cyclodextrins

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ARTICLE INFO

Article history:
Received 7 February 2013
Received in revised form 24 April 2013
Accepted 19 May 2013
Available online 6 June 2013

Keywords:
Anticancer drug
Topotecan
Cyclodextrin
Spectroscopy
Inclusion complexes
Proton-transfer dynamics

ABSTRACT

We report on photophysical studies of the interaction between an anti-cancer drug, topotecan (TPT), in aqueous buffered (pH = 7.23) solutions of three different β-CDs, native and methylated ones (DM-β-CD and TM-β-CD). We used UV–visible absorption and emission (steady-state and time-resolved) spectroscopy to follow the dynamical and structural changes due to the hydrophobicity and confinement effect of the CDs on both the ground- and excited-state behaviour of TPT. Both ^{1}H NMR and absorption experiments give evidences for the encapsulation of the enol form of TPT as the most favourable one. In addition, the host–guest interaction becomes stronger as the hydrophobic character provided by the methylated groups of the host increases. The equilibrium constants of the formed TPT:β-CD, TPT:DM-β-CD, and TPT:TM-β-CD 1:1 complexes are $K_{296\,K}$ ($10^4\,M^{-1}$) = 0.88, 2.4, and 3.7, respectively. Semiempirical (PM3) calclulations suggest that the docking of TPT is through its quinoline moiety, in agreement with the ^{1}H NMR assignment. We found that the hydrophobic environment provided by the CD cavity influences the deactivation channels of the emitting species by modifying the rate of the non-radiative processes upon encapsulation. The excited-state proton-transfer (ESPT) rate constants are affected by the degree of protection of the guest inside the host. Rotational times from picosecond anisotropy measurements (φ = 156, 169, and 178 ps for TPT, TPT:β-CD, and TPT:TM-β-CD, respectively) indicates that the drug is still able to rotate inside the CD. These findings are relevant to drug–host interactions and proton-transfer reaction dynamics in supramolecular systems for structurally related drugs, and should contribute to the development of drug delivery field.

1. Introduction

Topotecan (TPT, Fig. 1) is a semi-synthetic camptothecin (CPT) derivative, one of the human topoisomerase I (Top1) enzyme inhibitors leading to the lethal DNA strand break [1]. The H-atoms on the 9- and 10-positions of the i-ring in CPT are replaced by a dimethylaminomethylene and a hydroxyl (OH) group, respectively, to give TPT. Compared to CPT, TPT has a higher solubility in water and a lower cytotoxicity in human tissues [2]. TPT has been approved for oral use as an anti-cancer drug for the treatment of several cancers [3–5].

It is well known that CPT and, to some extent, its derivatives, are not stable at physiological conditions, where they undergo a pH-dependent, reversible hydrolytic rupture of their lactone function (v-ring) to give a relatively inactive carboxylate form in aqueous solution (Scheme 1S in Supplementary Data) [6,7]. One strategy to overcome the hydrolization process in CPTs is to create a host–guest system between the drug and an appropriate nanocarrier [22–25].

The ability of cyclodextrins (CDs) to encapsulate organic and inorganic molecules has led to intensive studies of their inclusion complexes as drug delivery nano-carriers [8–18]. These studies were devoted to understand and control the photophysical and photochemical behaviour of the molecular guests (emission enhancement/reduction, excimer/exciplex formation, photocleavage, charge- and proton-transfer, energy transfer, and cis-trans photoisomerization) for a better drug design and delivery. The effects of molecular confinement in terms of cavity size of the host and protection of the guest provided by the hydrophobic pocket of the CD have been found to play a key role on both the ground- and excited-state behaviours of the encapsulated molecule [8–18]. For example, encapsulation of Levosimendan (LSM), a cardiovascular calcium sensitizer, by β-CD leads to a robust 1:1 complex which displays an increase in its emission lifetime (from ~1.2 ps in water solutions of pH 7 to 4 ps within the β-CD cavity) [10]. On the other hand, the formation of 1:1 ($K_{11} = 199 - 501\,M^{-1}$), 1:2 ($K_{12} = 6.3 \times 10^7\,M^{-2}$), 2:1 ($K_{21} = 2.5 \times 10^4 - 7.9 \times 10^4\,M^{-2}$), and 2:2 ($K_{22} = 3.0 \times 10^{10}\,M^{-3}$) complexes of doxorubicin (DOX), a

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http://dx.doi.org/10.1016/j.jphotochem.2013.05.011

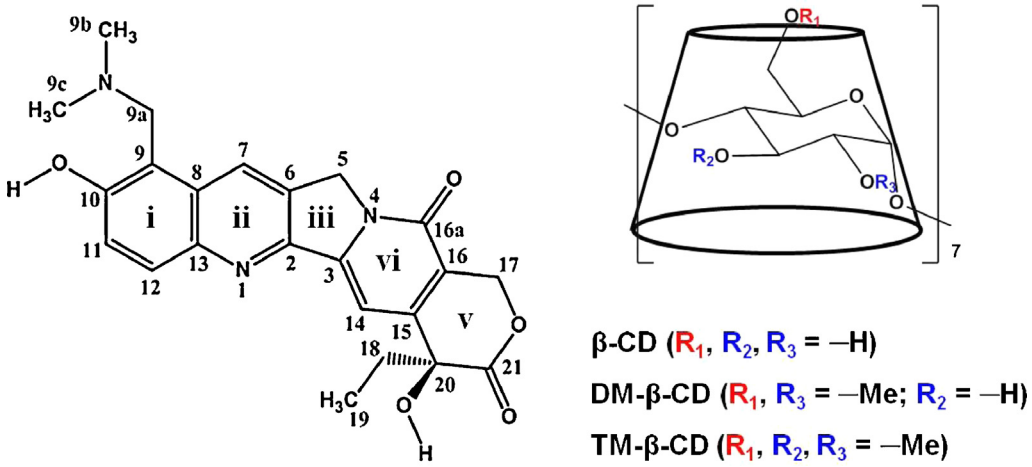

Fig. 1. Molecular structures of topotecan (TPT) in its lactone form and of CDs used in this work: β-CD, DM-β-CD, and TM-β-CD.

chemotherapic agent, with γ-CD has been investigated by the use of steady-state (UV–visible absorbance and emission spectra, circular dichroism) techniques in a wide range of DOX concentrations [15]. The drug release from the CD complex is mostly due to its dilution and interaction with the fluid components. Several efforts are devoted to improving the bioavailability of the drug by controlling its release in the body [19]. In this regard, CD-based polymeric carriers encapsulating strongly hydrophobic molecules like DOX and artemisinin (ART) have shown promising results which open the way to further design of novel poly-CD nanoassemblies as drug carriers [18].

In previous studies [20,21], we have reported on the steady-state (UV–visible absorption and emission) and time-resolved picosecond (ps) emission studies of TPT in organic solvents (THF, DCM, ACN, and MeOH) and in aqueous solutions of different pHs (pH = 0.48–12.15). The results show the effect of the H-bonding surroundings and the proton concentration on the structural and dynamical properties of TPT at both its ground- and excited-states. In addition to that, supramolecular inclusion complexes of TPT into liposomes, CDs (native and hydroxypropylated β-CD), and sulfonatocalix[4]arene (SC4A) have been investigated by means of high pressure liquid chromatography (HPLC), differential scanning calorimetry (DSC), and spectroscopic (UV–visible absorption and emission spectra, two-dimensional proton nuclear magnetic resonance (^{1}H NMR)) methodologies [22–25]. The results show an increase of the solubility, stability, and bioavailability of the encapsulated drug with respect to its free form [22,23,25]. However, as far as we know, there is no direct information on the topology and excited-state dynamics of the drug upon interaction with CDs.

Thus, here we report on the confinement effect of regular and several modified CDs (β-CD, heptakis(2,6-di-O-methyl)-β-CD (DM-β-CD), and heptakis(2,3,6-tri-O-methyl)-β-CD (TM-β-CD), Figs. 1–2) on the photophysics of TPT in buffered solution at physiological conditions (pH = 7.23). Our efforts are aimed at understanding the nature of the formed complexes and the influence of the CD nanoconfinement on the behaviour of caged TPT at its ground- and electronically first excited-states, using steady-state and time-resolved spectroscopic techniques. We found that the CD environment influences the deactivation channels of the emitting TPT species, modifying the rate of the non-radiative processes upon its encapsulation. Furthermore, ^{1}H NMR and semiempirical (PM3) calclulations are employed to support the discussion on the docking process to form the TPT:CD complexes. These findings give direct information of TPT, an anti-cancer drug, interacting with the CD nanocavity for drug storage, carrier, and delivery.

2. Experimental

TPT ((S)-(+)-topotecan hydrochloride) (Sigma–Aldrich, ≥98%), β-CD (β-cyclodextrin) (Acros Organics, >99%, H_2O ∼0.8%), DM-β-CD (heptakis(2,6-di-O-methyl)-β-cyclodextrin, degree of substitution (DS) ∼14 for Me, 7 on the small gate, 7 on the big gate) (CycloLab, >95% for methylated β-CDs, H_2O ∼0.7%), TM-β-CD (heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin, DS = 21 for Me, fully substituted) (CycloLab, >98%, H_2O ∼1.5%), anhydrous tetrahydrofuran (THF) (Sigma–Aldrich, ≥99.9%), and deuterium oxide (D_2O) (Sigma–Aldrich, >99%) were used without further purification. A phosphate ($NaOH/KH_2PO_4$) buffered solution (pH = 7.23) was used in all the cases. The buffered solution was prepared using doubly distilled water. The relative error of the measured pH was estimated to be around 2%. The TPT solutions were prepared at the concentration of $\sim 10^{-6}$ M except for the NMR experiments, where 10^{-5} M solutions in D_2O were used. The concentration of the β-CDs solutions was in the range 10^{-5} to 10^{-4} M except for the NMR experiments, where 10^{-2} M solutions of β-CD and TM-β-CD in D_2O were used. Steady-state absorption and emission spectra were recorded on a Jasco V-670 spectrophotometer and or Fluoromax-4P spectrofluorimeter, respectively. The emission spectra of TPT in presence of CDs were corrected for the fraction of light absorbed by the complex. Emission quantum yields were calculated using a solution of quinine sulphate in 0.5 M H_2SO_4 ($\Phi_F^{345} = 0.546$) as a standard [26]. The excitation wavelength for both the standard and the TPT molecule was 371 nm. The relative error of the measured emission quantum yields was estimated to be ∼10%. Steady-state fluorescence anisotropy (r) experiments were carried out on a Perkin–Elmer (LS-50B) spectrofluorimeter. The sample was excited at 371 nm, and the signal was gated at the emission intensity maximum. Each anisotropy value is an average of three independent measurements. Emission lifetimes were measured by using a picosecond (ps) time-correlated single-photon-counting (TCSPC) spectrophotometer (FluoTime 200, PicoQuant) described elsewhere [20]. The sample was excited by a 40 ps pulsed (20 MHz) diode laser, centred at 371 or 433 nm. The instrumental response function (IRF) of the apparatus was typically 65 ps. The fluorescence signal, gated at magic angle (54.7°), was monitored at a 90° angle to the excitation beam at discrete emission wavelengths. Decay data were analyzed using the FluoFit software package (PicoQuant). Exponential decay functions were convoluted with the experimental response function and fit to the experimental decay. The shorter component which could be resolved after a convolution process has a decay time of 15 ps. The quality of the fits as well as the number of