Chemistry and Physics of Lipids 210 (2018) 122–128

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

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

Shipment of a photodynamic therapy agent into model membrane and its controlled release: A photophysical approach

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ARTICLE INFO

Keywords:
Absorbance
Fluorescence
Photodynamic therapy
Structural switchover
Drug release

ABSTRACT

Harmine, an efficient cancer cell photosensitizer (PS), emits intense violet color when it is incorporated in well established self assembly based drug carrier formed by cationic surfactants of identical positive charge of head group but varying chain length, namely, dodecyltrimethylammonium bromide (DTAB), tetradecyltrimethylammonium bromide (TTAB) and cetyltrimethylammonium bromide (CTAB). Micelle entrapped drug emits in the UV region when it interacts with non-toxic β-cyclodextrin (β-CD). Inspired by these unique fluorescence/structural switching properties of the anticancer drug, in the present work we have monitored the interplay of the drug between micelles and non-toxic β-CDs. We have observed that the model membranes formed by micelles differing in their hydrophobic chain length interact with the drug differently. Variation in the surfactant chain length plays an important role for structural switching i.e. in choosing a particular structural form of the drug that will be finally presented to their targets. The present study shows that in case of necessity, the bound drug molecule can be removed from its binding site in a controlled manner by the use of non-toxic β-CD and it is exploited to serve a significant purpose for the removal of excess/unused adsorbed drugs from the model cell membranes. We believe this kind of β-CD driven translocation of drugs monitored by fluorescence switching may find possible applications in controlled release of the drug inside cells.

1. Introduction

Over 25 years of preclinical and clinical studies worldwide have established Photo Dynamic Therapy (PDT) as an efficient treatment approach against some cancer. Photofrin, a hematoporphyrin derivative compound, is most commonly used in the photo chemotherapy or photodynamic therapy since 1993 (Dougherty et al., 1998). The photochemical and photophysical processes in the photosensitizer (PS) during PDT are the key to the generation of reactive oxygen species (ROS). When a PS in its ground state is exposed to light of a specific wavelength, it absorbs a photon and is promoted to an excited singlet state. The singlet state is eventually decayed to the triplet excited state via intersystem crossing (ISC) and then the triplet state energy is transferred to ground state molecular oxygen to produce singlet oxygen. It is the cytotoxicity of the singlet oxygen that can cause oxidation of biomolecules and, finally, cell death. The singlet oxygen is promised to be highly efficient in treating cancer because of its short lifetime (< 0.04 μs) and short radius of action (< 0.02 μm) (Blum, 1941). The enhancement in the ROS generation can essentially increase the overall activity of a PS; thereby reducing the concentration of the essential photosensitizer in PDT.

The efficacy of the photodynamic action depends greatly on the structural aspects of the PS. As a matter of fact the structural form of these biologically active molecules are very much correlated with their function (Varela et al., 2001; Dias et al., 1996). β-Carboline alkaloids (Varela et al., 1995) and their derivatives (Cao et al., 2005a) are counted as benevolent photosensitizers and act upon photoexcitation by UVA (Gonzalez et al., 2010, 2012a,b). Studies in living cells have revealed that molecules belonging to this class exist both in neutral and protonated forms in cytoplasm, but only in its protonated form in the nucleus (Varela et al., 2001; Dias et al., 1996). Recently, triplet state studies on some β-carboline molecules by Varela et al. (2001) revealed that in their neutral forms, these compounds have significant triplet state yield and the long-lived triplet states may play important role in their photosensitization reactions in vivo in presence of oxygen. Under the situation, it is very much logical to assume that for a particular prototropic probe, it is often necessary to opt for one prototropic form or a desired composition of the different prototropic species for achieving better efficiency for a targeted purpose in a specific environment. Several studies are undertaken to establish the structure

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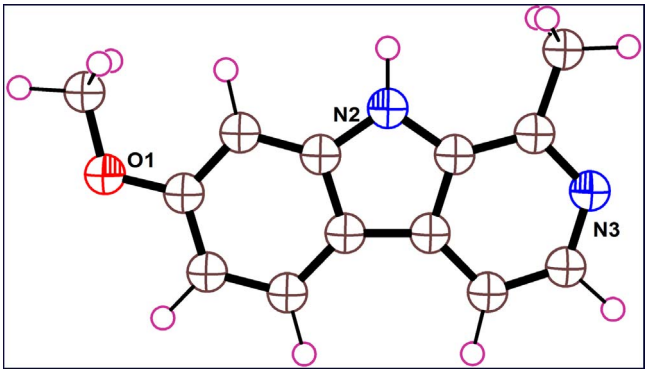
http://dx.doi.org/10.1016/j.chemphyslip.2017.10.005
Received 3 July 2017; Received in revised form 25 September 2017; Accepted 18 October 2017
Available online 19 October 2017
0009-3084/



Scheme 1. Structure of HM molecule.

activity relation (SAR) of β-Carbolines with variety of substituents at different positions (Blum, 1941; Varela et al., 2001, 1995; Dias et al., 1996; Cao et al., 2005a; Gonzalez et al., 2010, 2012a,b). Studies reveal that the protonated form of the β-Carbolines is responsible for most of the DNA damage (Vignoni et al., 2013). It is also well documented that β-Carbolines are able to damage chromosomes in mammalian cells (Mori et al., 1998), acts as antibacterial (Shimoi et al., 1992) and also against viruses (Hudson et al., 1986; Song et al., 2014) and antifungal activities (Song et al., 2014). β-Carbolines are family of alkaloids consisting of planer aromatic 9*H*-pyrido[3,4-*b*]indole structure found in many plants (Kam and Sim, 1998), arthropods (Stachell et al., 1999) and insects (Siderhurst et al., 2005). It is endogenously synthesized in mammals (Susilo and Rommelspacher, 1987) and its concentration elevates after post alcohol consumption and smoking (Breyer-Pfaff et al., 1996).

Harmine (HM) (Scheme 1), a member of the β-Carboline family, is fully aromatic, isolated from the Middle Eastern grass harmal or Syrian rue (*Peganum harmala*) and South American vine ayahuasca (*Banisteriopsis cappi*) (Becker and Sippl, 2011; Wegiel et al., 2011). Extensive research reveals that HM is strongly associated with anticancer activity (Cao et al., 2005a; Xiao-Juan et al., 2016; Liu et al., 2016; Filali et al., 2016; Martín et al., 2004; Frédérick et al., 2012). HM plays active role in tumor proliferation, inducing apoptosis (Xiao-Juan et al., 2016). Studies suggest that HM inhibits protein kinase DYRK1A (dual-specificity tyrosine-phosphorylated and regulated kinase 1A) *in vitro*. (Gockler et al., 2009) inducing the activation of caspase-9 leading to massive apoptosis in a number of human cell types and melanomas that are intrinsically resistant to apoptotic stimuli (De Wit et al., 2002). Overexpression of DYRK1A is associated with manifestation of several diseases including tumorigenesis (Laguna et al., 2008) and also lead to the cognitive deficits in people with Alzheimer's disease and Down syndrome (Smith et al., 2012). Interestingly, recent studies show that HM can reverse the anticancer drug resistivity of cancer cells by inhibiting the breast cancer resistance protein (BCRP) (Ma and Wink, 2010). Cao et al. studied DNA-HM binding properties and devised cytotoxic assay not only with HM but also with its derivatives. They further reported that HM and its derivatives show significant activities towards DNA intercalation capacity and inhibition of topoisomerase I but not topoisomerase II (Cao et al., 2005b).

Till date, adverse drug reactions (ADR) remain a serious problem in spite of considerable time and effort have been invested in this research (Stevens, 2006). Studies pertaining drug metabolism and pharmacokinetics (DMPK) play vital role in the discovery and sustainability of drugs (Buch, 2010). Undesirable adverse toxicity, post-clinical intervention along with compromised drug efficacy still counted as a major reasons behind several failures (Stevens, 2006; Buch, 2010).

Another most important issue of drug administration is the post-treatment side effects. Two most popular way outs frequently counted are (a) controlled delivery of drugs (Biswas et al., 2016; Reddy et al., 2015; Hirayama and Uekama, 1999) and (b) flushing/nullifying/detoxifying of excess drugs (Ghosh et al., 2014a). Cyclodextrins, their derivatives and other outer-hydrophilic-inner-hydrophobic molecules and macromolecules have been proven to be efficient drug carriers with on-demand triggered release mechanisms (Rajendiran et al., 2016). There are a number of reports on this kind of controlled drug delivery. But in the literature, there is feeble amount of reports on efficient recovery of excess drug. In addition, this is related to delocalized/non-specific drug distribution and unintentional drug overdose. This is more relevant towards cancer and related complicacies that demand highly site-specific treatment. Distribution of cancer drugs must be extremely site specific to minimize the post therapeutic side effects.

Micelles are the most extensively used membrane mimetic systems as delivery agents for drugs and genes. In addition, micelles have been very successfully utilized for triggered release, trafficking, optimizing availability of desired chemical species, signaling, sensing of ions, molecular recognition and creating favorable environment for reactions that are not energetically favorable in homogeneous medium (Fendler, 1982; Muller, 1973; Rammurthy, 1991). Our present work utilizes the micellar medium as delivery agent for HM, whereas β-CD has been employed as a drug capturing agent with an aim to develop a targeted drug delivery system. Site-directed drug delivery is the need of the time and the efficacy of delivery depends on the matching properties of delivery and capturing media. The choice of micellar medium as the deliver agent over lipid vesicles is because of its easy tunablity, so that an environment of chosen hydrophobicity can be easily designed. Nonetheless, the lipid vesicles could be used as delivery agent but partition coefficient of HM between lipid vesicles and β-CD will be different as the environmental hydrophobicity of lipid membranes and micelles are different.

2. Experimental section

HM and β-CD were procured from Aldrich (Missouri, USA) and used as received. All the surfactants, namely, DTAB, TTAB and CTAB were procured from Lancaster (England) and used as received. Spectroscopy grade water from Millipore was used throughout the experiment. Hitachi U5300 spectrophotometer (Tokyo, Japan) with thermostated cell holder & stirrer was employed to measure the absorption data. All steady-state fluorescence experiments were carried out on Hitachi F7000 spectrofluorometer (Tokyo, Japan).

HM stock solution was prepared by dissolving 1.5 mg/mL in DMF through proper sonication. To achieve the desired concentration, stock surfactant solutions with sufficiently high concentrations were gradually added directly to the probe solution in the quartz cuvette. Volume fractions were kept below 5 μL so that the addition process practically did not change the probe concentration. The total solution in the quartz cuvette was properly stirred on a magnetic stirrer. Then after proper thermal equilibration, the spectra were recorded. Because of very low solubility of β-cyclodextrin in water (18.5 mg/mL), pre-weighted solid β-CD were added to the probe-surfactant mixture directly in the cuvette and stirred on a magnetic stirrer for a sufficient time to achieve a homogeneous thermally equilibrated solution with desired β-CD concentration and proceed for the spectral measurements. All the experiments were performed at ambient temperature (300 K) with air-equilibrated solutions.

Fluorescence lifetimes were determined in degassed solution of the probe from time resolved intensity decay by the method of time correlated single-photon counting (TCSPC) using a 300 nm nanoLED (IBH U.K.) as the light source. The typical response of this excitation source was 1.2 ns. The decay curves were analyzed using IBH DAS-6 decay analysis software. We fitted the lifetime data with a minimum number of exponential. Goodness of fit was evaluated by χ^2 criterion and visual inspection of the residuals of the fitted function to the data. The value of $\chi^2 \approx 1$ was considered as the best fit for the plots. The lifetimes were measured in air-equilibrated solution at ambient temperature.