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Preparation, characterization, and in vitro phototoxic effect of zinc phthalocyanine cucurbit[7]uril complex encapsulated into liposomes

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ABSTRACT

Inclusion complexes improve the solubility, dissolution, and bioavailability of hydrophobic drugs. In this study, we used the cucurbit[7]uril (CB[7]) to improve the solubility of zinc phthalocyanine (ZnPc) in water. The CB[7]:ZnPc complex was prepared by co-evaporation, characterized it by ^{1}H NMR spectroscopy, differential scanning calorimetry, X-ray diffraction, UV–vis spectroscopy, and molecular simulation. Next, the CB[7]:ZnPc inclusion complex was incorporated into liposomes (CB[7]:ZnPc-Lp) to obtain a drug delivery system and assess the in vitro phototoxicity of both free and liposomal CB[7]:ZnPc on B16-F10 melanoma cells. ^{1}H NMR confirmed that the CB[7]/ZnPc proportion in the inclusion complex was 1:1, as corroborated by X-ray diffraction, differential scanning calorimetry, and UV–vis spectroscopy. Molecular simulation evidenced that the structures of ZnPc and CB[7] interact in the inclusion complex. The thin lipid film method with subsequent extrusion furnished liposomes containing CB[7]:ZnPc compared with the free inclusion complex, CB[7]:ZnPc-Lp exerted excellent phototoxic effect on melanoma cells. In conclusion, inclusion complexes effectively solubilize hydrophobic compounds, such as zinc phthalocyanine and allow for their incorporation into liposomes, generating excellent drug delivery systems.

1. Introduction

Macrocyclic host molecules can encapsulate biologically relevant guests and act as drug carriers, drug solubilizers, drug stabilizers, and drug bioavailability enhancers [1]. Cucurbit[n]urils (CB[n]) are a family of cyclic host molecules consisting of glycoluril units linked by a pair of methylene groups. They possess fairly rigid hydrophobic cavities of low polarizability that can be reached through carbonyl-lined portals [2]. The improved syntheses of CB[n], and in particular the superior solubility of CB[7] in aqueous solution, have prompted investigations into host–guest behavior of CB[n] with a variety of aromatic and metal complex guest molecules [3,4] in water, including bioactive species such as oxaliplatin and other anticancer platinum complexes [5]. Like β-cyclodextrin, the cavity of CB[7] can accommodate aromatic molecules; this cavity has a portal diameter and an internal cavity diameter of 5.4 and 7.3 Å, respectively [6]. CB[7] is a symmetric compound that contains seven substituents (see structure in Fig. 1(A)); their respective carbons present hydrogens numbered 1 to 4. Entrapping drug inclusion complexes into drug delivery systems, such as liposomes [7,8] or nanoparticles [9], prevents these complexes from dissociating, and alters the pharmacokinetics of the target drug.

Photodynamic therapy (PDT) harnesses the power of light, photosensitizer drug (PS), and oxygen to enact biological change. In the beginning, on account of the prolonged and pronounced photosensitivity resulting from systemic photosensitizer agents, the use of PDT to treat skin diseases was limited [10]. An ideal PS should (i) be pure and has constant composition and stable shelf life, (ii) be water soluble or soluble in a harmless aqueous solvent mixture, (iii) not aggregate unduly in biological environments, because aggregation reduces photochemical efficiency, and (iv) be rapidly eliminated from the patient's body (in less than one day) to dismiss the need for post-treatment protection from light exposure avoiding prolonged skin photosensitivity [11]. Phthalocyanines containing metals into its macrocycle have long been used as blue-green dyes and pigments. Zinc phthalocyanine (ZnPc) is a

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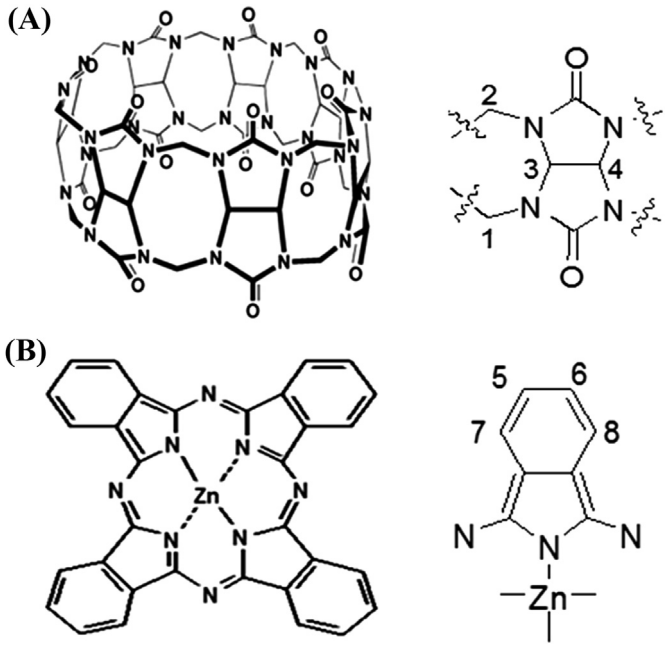

Fig. 1. Chemical structure of cucurbit[7]uril (A) and zinc phthalocyanine (B) with atom assignments.

porphyrinoid photosensitizer belonging to the *second-generation* sensitizer drugs which exhibits low dark toxicity and excellent thermal and chemical stability. ZnPc is symmetric and bears four identical substituents (Fig. 1(B)); the respective carbons contain hydrogens numbered 5 to 8. Such metallophthalocyanine demonstrate high affinity for cancer cells and tumor tissues; however, ZnPc is a well known lipophilic dye and its insolubility in water has hampered the pharmaceutical formulation development as well as directly application in biological medium. In addition, hydrophobic metallophthalocyanines aggregate easily in aqueous medium forming photochemically inactive PS [12–14]. Therefore, to overcome this limitation an inclusion complex from ZnPc and cucurbituril cyclic host molecules and/or its encapsulation into liposomes is highly recommended [12,14,15].

In this work, we prepared an inclusion complex between ZnPc and cucurbit[7]uril and characterized the resulting CB[7]:ZnPc complex in terms of ^{1}H NMR spectroscopy, differential scanning calorimetry, X-ray diffraction, UV–vis spectroscopy, and molecular simulation. In addition, we encapsulated CB[7]:ZnPc complex into liposomes and assessed the in vitro phototoxic effect of free CB [7]:ZnPc and CB[7]:ZnPc-Lp on melanoma cells. Therefore, the aim of this study was develop an innovative cucurbituril-complexed ZnPc able to improve the solubility in water of this PS and incorporate it into liposomes allowing its application in PDT protocols against skin cancer.

2. Materials and methods

2.1. Materials

Zinc phthalocyanine (ZnPc) (97%), stearylamine (98%), methanol (MeOH), dimethyl sulfoxide (DMSO), and dimethylformamide (DMF) were obtained from Sigma (Sigma–Aldrich Co., St. Louis MO, USA). Cholesterol and L-α-phosphatidylcholine (egg chicken) were purchased from Avanti (Avanti Polar Lipids, Alabama, USA). Cucurbit[7]uril (CB[7]) was prepared and purified according to the method described by Day et al. [16].

2.2. Preparation of CB[7]:ZnPc inclusion complex

The solid CB[7]:ZnPc inclusion complex was prepared in a 1:1 molar ratio (0.5×10^{-3} mol), using co-evaporation method. Cucurbit[7]uril and zinc phthalocyanine were dissolved in 60 mL of 50% (v/v) aqueous ethanol at 40 °C. The solution was stirred for 24 h, at room temperature. The solvent was removed in a rotary evaporator, at 40 °C. The mixture was frozen, and water was removed by lyophilization [17].

2.3. Characterization of CB[7]:ZnPc inclusion complex

2.3.1. ^{1}H NMR spectroscopy

CB[7], ZnPc, and CB[7]:ZnPc were characterized by ^{1}H NMR analysis using a Bruker Avance DRX-500 instrument. The samples diluted in deuterated dimethyl sulfoxide (d6-DMSO). Chemical shifts (δ) are reported in parts per million (ppm) relative to the internal standard tetramethylsilane (TMS).

2.3.2. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) thermograms of CB[7], ZnPc, and CB[7]:ZnPc (3 mg) were acquired on a DSC-50 thermal analyzer (Shimadzu, Japan), using 50 mL min^{-1} nitrogen flow rate, at temperatures ranging from 150 to 450 °C and a heating rate of 10 °C min^{-1}. All the samples were placed in hermetically sealed aluminum pans. An empty aluminum pan was used as reference.

2.3.3. X-ray powder diffraction

The powder X-ray diffractograms of the samples were recorded on the Siemens D5005 model (now Bruker AXS). The samples were placed in a sealed Cu tube (K (α radiation)) of 1.5406 Å, with the 2θ angle ranging from 5 to 40 °C.

2.3.4. Absorption spectroscopy UV–vis

Spectrometric measurements were carried out in the range of 300–800 nm with a UV–vis system model Perkin Elmer Lambda 20. From the stock solution of ZnPc (200 μg mL^{-1}) in DMF/DMSO (1:1), absorption spectra were read in a final concentration of 5.2 μg mL^{-1}. The absorption studies were performed in organic medium and in the presence of increasing water concentration in the mixture. Finally, the spectra were compared with those recorded for the inclusion complex prepared in aqueous medium as described in Section 2.2. Quartz cells with 1 cm optical path were employed in these assays.

2.4. Molecular simulation

The structures of CB[7] and ZnPc were originally designed with the programs ChemSketch and VegaZZ. The equilibrium geometries of each molecule were found using methods based on the quantum Density Functional Theory (DFT). The inclusion complex CB[7]:ZnPc was assembled starting from the optimized geometries of CB[7] and ZnPc; the geometries were optimized using quantum methods based on DFT and the program Jmol (An Open Science Project), which enabled visualize the complex and observe changes in the distances between the molecules before and after the inclusion complex was formed. Calculations were performed in vacuum (no solvent). Dr. Luis Gustavo Dias from FFCLRP (Universidade de São Paulo, Ribeirão Preto/SP, Brazil) calculated the molecular structure of the inclusion complex.

2.5. Preparation of liposomes containing CB[7]:ZnPc complex

Liposomes containing the CB[7]:ZnPc complex (CB[7]:ZnPc-Lp) were prepared by the thin lipid film method [18]. Briefly,