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Interaction of Riboflavin-5-Phosphate With Liposome Bilayers

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ABSTRACT

Riboflavin presents tremendous potential as a photosensitizing agent for photodynamic therapy (PDT) for treating microbial infection and cancer therapy. Encapsulation of riboflavin can improve its bioavailability and stability while making the clinical applications more efficient. The authors' detailed study on cellular inhibition of liposome encapsulated riboflavin-5-phosphate investigation, and the effect of unencapsulated riboflavin on liposome bilayers aims to improve the efficiency of cellular delivery of riboflavin. Nano-sized liposomes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol were used in this study. Cell studies demonstrate high inhibition rates for the lipsome-encapsualted high concentration riboflavin formulations in the presence of blue light, despite the lower encapsulation lading.

KEYWORDS

Differential Scanning Calorimetry, DPPC, Liposomes, Photodynamic Therapy, Riboflavin

INTRODUCTION

A beam light at a visible or near infrared (NIR) wavelength to destroy the target cells bases photodynamic therapy (PDT) or a photodynamic reaction involving a light-sensitive substance (a photosensitizer) combined with the irradiation. It is emerging as a highly effective, non-invasive therapeutic approach in the struggle against cancer and other infectious diseases (Ma, Huang, Song, Chen, & Zhang, 2016). Despite the significant progress and scientific reports, PDT is yet to be established as an effective and safe technique to eradicate microbes and tumors (Konan, Gurny, & Allemann, 2002). Riboflavin-5-phosphate, also referred as vitamin B_2, is a potent antioxidant and is used as a supplement in chemotherapy due to its anti-carcinogenic properties (Ashoori & Saedisomeolia, 2017; Kandzija & Khutoryanskiy, 2017). In addition to the anti-carcinogenic properties, riboflavin has also reported as a potential photosensitizer for PDT. The photosensitive property of riboflavin has been investigated in eliminating tumor, ocular and skin and bacterial infections (Arboleda et al., 2014; Edwards, Barredo, Silva, De Ioannes, & Becker, 1999; Ion, 2007; Kashiwabuchi ., 2012; Khaydukov et al., 2016; Sato, Sakakibara, Hasegawa, Minami, & Tsuji, 2000; Shen ., 2017). However, the hydrophilic nature of riboflavin causes rapid clearance of the drug in the blood stream and lowers the intracellular absorption, thereby, reducing its therapeutic efficacy (Eloy et al., 2014). Liposome based carriers have been exploited to encapsulating hydrophilic drugs to prevent their rapid clearance and increase their circulation time upon administration. (Eloy et al., 2014; Pabst, Kučerka, Nieh, & Katsaras, n.d.; Torchilin, 2005; Xu, Khan, & Burgess, 2012).

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Liposomes are self-assemblies of lipids, which are amphipathic in nature consisting of a hydrophilic head and a hydrophobic tail. Liposomes are characterized by a lipid bilayer surrounding aqueous core which self-assemble to give rise to an aqueous core (Gupta, Mandal, Ahmadibeni, Parang, & Bothun, 2011; Matos, Moutinho, & Lobão, 2012). Due to this unique structure, they are capable of entrapping both hydrophobic and hydrophilic molecules (Murthy, 2007). A variety of simple and economic methods such as dry film hydration, solvent exchange, electro formation methods have been investigated to form liposomes with variable size, surface charge, and number of bilayers (Patil & Jadhav, 2014). Addition of cholesterol improves the circulation of liposomes in the blood stream while providing the steric stabilization through increasing the rigidity of the bilayer (Tan, 2015). Although the efficacy of liposome encapsulated riboflavin-5-phosphate formulations has been reported (Ahmad et al., 2015; Ioniţă, Ion, & Cârstocea, 2003) the effects of interaction between the free-floating riboflavin-5-phosphate, a potent riboflavin derivative on the stability of liposome carriers remain unaddressed.

The objective of the current work is two folds, first to test the efficacy of liposome encapsulated riboflavin riboflavin-5-phosphate in the presence and absence of blue light for their potential application in photodynamic therapy against cancer and infectious diseases. Secondly, to gain insight on the interaction of unencapsulated riboflavin-5-phosphate at higher concentrations on the liposomal bilayers. Accordingly, a combination of analytical studies such as zeta potential, DSC (Differential Scanning Calorimetry) were used to examine the electrostatic binding between the riboflavin and lipid headgroups. DSC was used to obtain information on the perturbations and disordering of the lipid bilayer due to the presence of riboflavin. A detailed quantification of thermodynamic properties associated with incorporation of riboflavin within the lipid bilayer was also conducted.

MATERIALS AND METHODS

Materials

DPPC (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine) dissolved in chloroform was purchased from Avanti Polar Lipids. Sigma Aldrich supplied cholesterol in powdered form. Riboflavin-5-monophosphate sodium salt (98% purity) was purchased from VWR. Invivogen Ultra-pure distilled water was used in the preparation of liposomes and riboflavin-5-phosphate solutions. Table 1 enlists the molecules used in this study along with their chemical structure and the properties.

Preparation of Liposomes

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), cholesterol and polycarbonate membranes were purchased from Avanti Polar Lipids (Alabaster, AL). DPPC/Cholesterol (90/10, 10mM) liposomes were prepared by mixing the DPPC and Cholesterol in known volumes. The mixture was left to dry under a stream of nitrogen followed by vacuum drying for 20 minutes. The dried lipid film was re-hydrated with *Invivogen* endotoxin free and the liposomes were extruded through 100 nm polycarbonate membranes to obtain unilamellar liposomes. To prepare the riboflavin-encapsulated liposomes, the dried lipid film was hydrated with 0.5 mM and 10 mM riboflavin-5-phosphate.

DLS and Zeta Potential Measurements

DLS (Dynamic Light Scattering) and Zeta potential experiments were recorded using Zetasizer nano-series (Malvern Nano-ZS). The size distribution of the liposomes was obtained by placing 1 ml of sample in SARSTEDT polystyrene cuvettes at a 173° backscatter angle with 120 s equilibration time. Zeta potential measurements were made using DTS1070 folded capillary cells. Size and zeta potential measurements were conducted at 25 and 43°C.