Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 173 (2017) 292–300

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Photophysical behavior and photodynamic therapy activity of conjugates of zinc monocarboxyphenoxy phthalocyanine with human serum albumin and chitosan

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ARTICLE INFO

Article history:
Received 19 May 2016
Received in revised form 24 August 2016
Accepted 18 September 2016
Available online 20 September 2016

Keywords:
Human serum albumin
Chitosan
Zinc monocarboxyphenoxy phthalocyanine
Singlet oxygen quantum yields
Dark cytotoxicity

ABSTRACT

Zinc monocarboxyphenoxy phthalocyanine (ZnMCPPc) was linked to human serum albumin (HSA) and chitosan *via* amide bond formation. The photophysical behavior and photodynamic therapy (PDT) activity (against human breast adenocarcinoma cell line (MCF-7 cells) of ZnMCPPc alone and its conjugates were investigated. The conjugates showed improved fluorescence, triplet and singlet oxygen quantum yields when compared to ZnMCPPc alone. The *in vitro* dark cytotoxicity and PDT studies were carried out at a dose of 3.6 μg/mL to 57.1 μg/mL. The *in vitro* dark cytotoxicity studies of ZnMCPPc showed cell viability <50% at 28.6 μg/mL and 57.1 μg/mL, while the conjugates showed > 50% in all their tested concentrations (3.6 to 57.1) μg/mL. Thus, conjugation of ZnMCPPc to HSA and chitosan improves its dark cytotoxicity, an important criteria for molecules meant for photodynamic therapy. Complex **1** showed the most efficacious PDT activity with cell viability <50% at concentration range of (14.3 to 57.1) μg/mL in comparison to the conjugates which only showed <50% cell viability at 28.6 μg/mL and 57.1 μg/mL for **1**-HSA and 57.1 μg/mL for **1**-Chitosan.

1. Introduction

Photodynamic therapy (PDT) is a minimally invasive treatment modality of tumor cells [1–3]. In PDT, the electronically excited photosensitizer transfers its energy to ground state molecular oxygen to produce excited singlet oxygen, the chief cytotoxic species which initiates an irreversible photo-damage of the tumor cells [4,5]. Phthalocyanines (Pcs) have been found to be viable photosensitizers for PDT due to their unique physicochemical properties such as, distinctive intense absorption in the red region of the visible spectrum and high singlet oxygen generation ability [4,5]. However, low solubility and aggregation limit the full applications of Pcs as photosensitizers. Drug delivery agents such as chitosan and serum albumins may be used to solubilize and reduce aggregation of Pcs. Chitosan is used extensively in drug delivery applications [6–11]. Chitosan (structure shown in Scheme 1) is a polysaccharide which contains primary amine groups, making it easy to link to other molecules through an amide bond. A porphyrin (similar in structure to a phthalocyanine) was covalently conjugated to chitosan nanoparticles for photophysical studies [12]. In another study magnetic chitosan nanoparticles were conjugated to 2,7,12,18-tetramethyl-3,8-di-(1-propoxyethyl)-13,17- bis-(3-hydroxypropyl) porphyrin to treat mice infected with human colon cancer [13]. Apart from studies on the catalytic behavior of phthalocyanines when supported on chitosan beads [14,15], there have been no studies on phthalocyanines linked (or supported on) chitosan for photophysical and PDT studies.

Serum albumins play an important role in the transport of many exogenous and endogenous ligands, binding covalently or reversibly to these ligands and increasing the passive tumor selectivity of the drug by enhanced permeation and retention effect [16–18]. Phthalocyanines have been linked mainly to bovine serum albumin (BSA) [19,20]. Low PDT activity of silicon phthalocyanine when conjugated to BSA was observed and this was explained to be due to increased aggregation tendency of phthalocyanine following BSA conjugation [19]. On the other hand, conjugation of chlorin e6 to human serum albumin followed by formation of self-assembled nanoparticle structures resulted in improved PDT activity [20]. A decrease in triplet yields of a zinc phthalocyanine derivative was observed in the presence of bovine serum albumin (BSA) [21]. Singlet state and/or static triplet quenching of the bound dye by BSA was suggested [21]. Thus, the effects of HSA/BSA on the photophysical behavior of phthalocyanines needs to be examined further. In this work a phthalocyanine is linked to human serum albumin (HSA) for photophysical and PDT studies.

The phthalocyanine employed in this work is zinc monocarboxyphenoxy phthalocyanines (ZnMCPPc, complex **1**, Scheme 1). The COOH group of ZnMCPPc will be linked to the amino group of HSA and chitosan to achieve amide bond formation. The photophysical behavior of the conjugates will be assessed. For PDT applications, photosensitizers should be non-toxic without light, thus it is important to evaluate the dark cytotoxicity of photosensitizers, hence this is

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http://dx.doi.org/10.1016/j.saa.2016.09.032
1386-1425/