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Synthesis of protoporphyrin–lipids and biological evaluation of micelles and liposomes

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ABSTRACT

Protoporphyrin IX (PPIX) lipids were synthesized by introducing a long alkyl chain, such as C13, C15, and C17, at each vinyl group on PPIX via hydrobromination. The PPIX lipids exhibited a water-soluble property by forming their micelles in water and the PPIX–lipid micelles showed relatively low cytotoxicity toward HeLa cells (IC_{50} = 151.7–379.9 μM) without light irradiation. PL-C17 liposomes (post-inserted liposomes) were readily prepared by adding PL-C17 micelle solution to the liposome solution. The IC_{50} values of PPIX, PL-C17 micelles, and PL-C17 liposomes toward HeLa cells were 0.53, 5.65, and 12.9 μM, respectively, after irradiation with a xenon lamp in the 400–800 nm range for 2 min. PL-C17 liposomes were selectively accumulated in the Golgi apparatus in cells.

1. Introduction

Photodynamic therapy (PDT) is a tumor treatment modality that uses a combination of photosensitizer, oxygen, and tissue-penetrating light.[1–4] Reactive oxygen species (ROS) generated from molecular oxygen through energy transfer of the photosensitizer induce significant oxidative damage to biomolecules, and cell death occurred as a result.[5] The selective accumulation of the photosensitizer in tissue and the subsequent light-irradiation dose applied to the target tissue are important issues for the successful application of PDT. Hematoporphyrin (HpD)[6] is the first photosensitizer to receive regulatory approval in Canada in 1993. Thereafter, HpD received approval also in the U.S., Europe, and Japan for the treatment of cervical, endobronchial, esophageal, bladder, and gastric cancers.[2] HpD is a mixture of compounds that include the hematoporphyrin monomer, dimer, and oligomers and Photofrin® (QLT PhotoTherapeutics Inc.), which consists of many porphyrin units, is the partially purified from HpD.[7] Benzoporphyrin derivative monoacid ring A, also known as Verteporfin™ (QLT PhotoTherapeutics Inc.), is a second-generation photosensitizer that has a longer wavelength for activation than photofrin.[8] However, as verteporfin is hydrophobic, it is necessary to change its formulations to enable delivery to the target tissue. Visudyne is the liposomal formulation of verteporfin and is used for the treatment of age-related macular degeneration.[9] Protoporphyrin IX (PPIX) is an important intermediate of biologically essential prosthetic groups, such as heme, cytochrome c, and chlorophylls, and 5-aminolevulinic acid (5-ALA), a naturally occurring amino acid, is a precursor of PPIX in the biosynthetic pathway. Although PPIX is converted into heme by the enzyme ferrochelatase, the activity of ferrochelatase is lower in certain tumors than in normal tissues, allowing the selective accumulation of PPIX in tumor after the administration of 5-ALA.[10] PPIX is also hydrophobic and 5-ALA-based PDT, where the accumulated endogenous PPIX acts as a photosensitizer, has received approval for the treatment of actinic keratosis, basal cell carcinoma, and Darier's disease.[11–13] Various classes of photosensitizers have been designed and synthesized so far, including porphyrins, phthalocyanines, chlorins, and non-porphyrin dyes.[1] However, most photosensitizers tend to be poor soluble in water. Therefore, much attention has been focused on drug-delivery vehicles as nanoscale drug-delivery platforms, such as liposomes.[14] Two strategies for the liposomal delivery of photosensitizers are available: (1) the encapsulation of water-soluble photosensitizers in the aqueous interior of liposomes (Fig. 1A), and (2) the accumulation of hydrophobic photosensitizers in the liposomal bilayer (Fig. 1B). We are interested in the development

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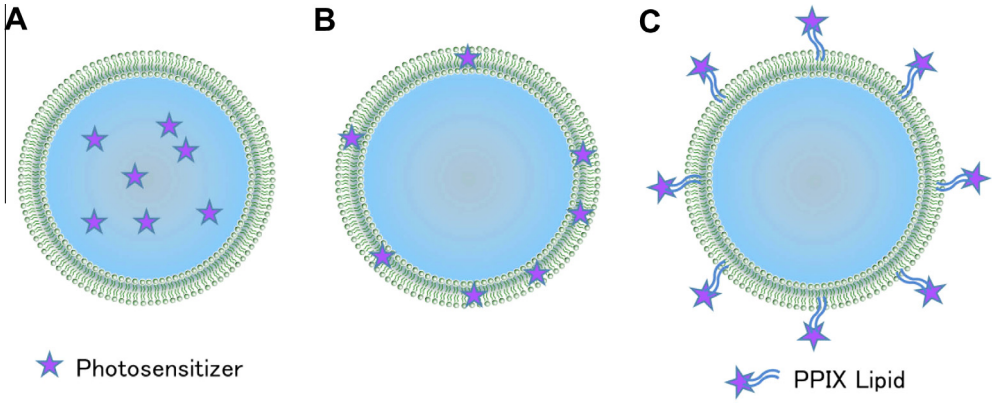


Figure 1. Strategies for liposomal delivery of photosensitizers. (A) Encapsulation of water-soluble photosensitizers in the aqueous interior of liposomes. (B) Accumulation of hydrophobic photosensitizers in the liposomal bilayer. (C) Post-insertion of PPIX lipids into liposomal bilayer (the approach used in this study).

of a water-soluble PPIX. To this end, we focused on the structure of PPIX and designed PPIX lipids by introducing a long alkyl chain to each vinyl group on PPIX via hydrobromination, in hopes that the PPIX lipids would form micelles in water. In this paper, we report the synthesis of PPIX lipids and their micelle formation. We also developed an alternative strategy for the liposomal delivery of photosensitizers, which involved the post-insertion of PPIX lipids into liposomal bilayer (Fig. 1C). Recently, phospholipid–porphyrin conjugates that exhibit a liposome-like self-assembled bilayer structure in a 100 nm-diameter nanovesicle, were developed for biophotonic imaging and therapy.[15,16]

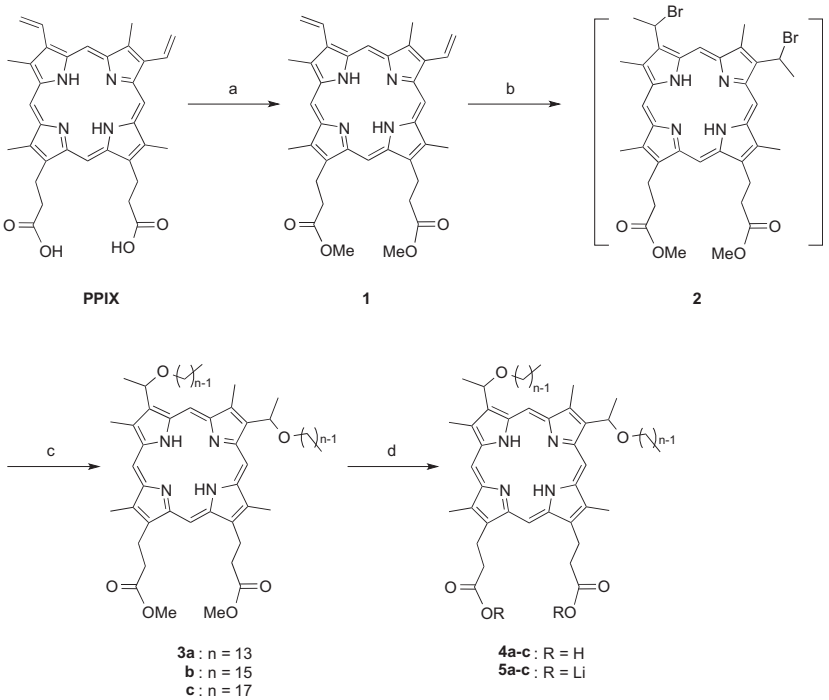
2. Results and discussion

2.1. Synthesis of PPIX–lipids

The synthetic route of PPIX–lipids is shown in Scheme 1. Acid esterification of protoporphyrin IX (PPIX; **1**) in the presence of H_2SO_4 in MeOH at −10 °C gave protoporphyrin IX dimethyl ester **2** in 95% yield. The vinyl groups of **2** were hydrobrominated with HBr/AcOH (25%) to yield the corresponding bis(α-methyl-β-bromo)protoporphyrin IX dimethyl ester **3**, which was then immediately treated with 1-tridecanol, 1-pentadecanol, and 1-heptadecanol in the presence of cesium carbonate (Cs_2CO_3) for 2 h at room temperature to give the corresponding ethers **4a–c** in 18–23% yields.[17] Finally, hydrolysis of **4a–c** with LiOH·H_2O in a mixed solvent system of THF/MeOH/H_2O (1:1:1) for 6 h afforded PPIX–lipids (PL-C13, PL-C15, and PL-C17) in high yields.

2.2. Characterization and dark cytotoxicity of PPIX–lipid micelles

PPIX lipids, PL-C13, PL-C15, and PL-C17, were dissolved in THF and aqueous lithium hydroxide solution was added. The resulting lithium salts of the PPIX lipids were dissolved in water to obtain their micelle solutions. Particle size (diameter) distributions of



Scheme 1. Synthesis of PPIX lipids. Reagents and conditions: (a) H_2SO_4, MeOH, −10 °C, 18 h, 95%; (b) HBr, AcOH, rt, 2 h; (c) ROH, CH_2Cl_2, K_2CO_3, rt, 2 h, **3a**; 23%, **3b**; 18%, **3c**; 21% in two steps; (d) (i) LiOH, MeOH/H_2O/THF (1:1:1), rt, 6 h; (ii) HCl (1 N), **4a**; 90%, **4b**; 90%, **4c**; 93%.