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Synthesis, photophysical properties and sugar-dependent in vitro photocytotoxicity of pyrrolidine-fused chlorins bearing *S*-glycosides

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ABSTRACT

Eight *S*-glycosylated 5,10,15,20-tetrakis(tetrafluorophenyl)porphyrins (**1a**′, **1b**′, **1a** and **1b** (**a**: *S*-glucosylated, **b**: *S*-galactosylated)) and their 1,3-dipolar cycloadducts, i.e. chlorins **2a**′, **2b**′, **2a** and **2b** were prepared by nucleophilic substitution of the pentafluorophenyl groups with *S*-glycoside. These photosensitizers were characterized by ^{1}H, ^{13}C and ^{19}F NMR spectroscopies and elemental analysis. The photocytotoxicity of the *S*-glycosylated photosensitizers and the parent porphyrin (**1**) and chlorin (**2**) was examined in HeLa cells. Photosensitizers **1**, **2**, **1a**′, **1b**′, **2a**′ and **2b**′ showed no significant photocytotoxicity at the concentration of 0.5 μM, while the deprotected photosensitizers **1a**, **1b**, **2a** and **2b** were photocytotoxic. The strong inhibition by sodium azide of the photocytotoxicity of these photosensitizers suggested that $^{1}O_2$ is the main mediator. The *S*-glucosylated photosensitizers **1a** and **2a** showed higher photocytotoxicity than *S*-galactosylated **1b** and **2b**, respectively. The cellular uptake of **1a** and **2a** increased up to 24 h, while that of **1b** and **2b** was saturated by 12 h.

1. Introduction

Photodynamic therapy (PDT) for cancer involves a photochemical reaction in tumor cells containing pre-administered photosensitizers and then exposed to light [1–6]. Photosensitizers generally have no cytotoxicity in the dark, but generate highly cytotoxic species, typically reactive oxygen species (ROS), when they are photoirradiated [2,6]. For clinical application, photosensitizers must meet several requirements: little or no cytotoxicity in the dark, selective accumulation in tumor cells rather than normal cells, photoabsorption in a long wavelength region (the so-called PDT window), and rapid clearance after treatment. Porphyrins and their reduced analogues, namely dihydroporphyrins (i.e., chlorins) and tetrahydroporphyrins (i.e., bacteriochlorins) have a number of advantageous photophysical and chemical properties for PDT, including a relatively large molar absorption coefficient at the PDT window.

In PDT, the photodynamic effect is determined by the distribution of the photosensitizer and the area of photoirradiation. The latter can be manipulated easily owing to recent developments in optical devices. In contrast, the distribution of photosensitizers is hard to regulate, and accumulation of photosensitizers in normal cells can lead severe side effects, even in dim light. Hence, tumor-selective accumulation of the photosensitizer is critical for successful PDT. One promising approach is conjugation of a photosensitizer with a biologically active element. This approach was utilized in the development of 2-[^{18}F]fluoro-2-deoxy-D-glucose for cancer diagnostics using positron emission tomography (PET), making use of the fact cancer cells show enhanced D-glucose uptake. Zhang et al. reported that a pyropheophorbide conjugated with glucosamine was preferentially taken up by tumor cells in glioma-bearing rats [7]. The inhibition of the cellular uptake of the glycoconjugated pyropheophorbide by addition of D-glucose suggested the participation of the GLUT/hexokinase pathway. Pandey et al. reported benzochlorin bearing glucosamine, galactosamine or lactosamine showed sugar-dependent photocytotoxicity towards radiation-induced fibrosarcoma (RIF) cells, which are known to express galectin-3 [8].

We systematically synthesized forty 5,10,15,20-tetraphenylporphyrin (TPP) and chlorin (TPC) derivatives bearing *O*-glycoside at the phenyl rings [9–13]. Our studies on *O*-glycosylated TPPs and TPCs revealed a number of advantages, including significant reduction of the dark cytotoxicity, improved water-solubility, greater

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cellular uptake, and sugar-dependent photocytotoxicity [9–13]. The *O*-glycosylated TPCs showed very promising photocytotoxicity in HeLa cells. However, it was difficult to purify *O*-glycosylated TPCs from the reaction mixture containing starting TPPs and tetraphenylbacteriochlorins [10]. Therefore, we decided to examine a different approach, such as Diels–Alder reaction or 1,3-dipolar cycloaddition, instead of diimide reduction. Cavaleiro et al. firstly reported Diels–Alder reaction of porphyrin **1** in which the porphyrin acts as a dienophile [14]. They also reported 1,3-dipolar addition of azomethine ylide to porphyrin **1** to afford a pyrrolidine-fused chlorin **2** [15,16]. Other types of 1,3-dipole such as nitrones [17], carbonyl ylide [18], diazomethane [19,20] and nitrile oxide [21] also yield fused chlorin. These fused chlorins formed by 1,3-dipolar cycloaddition are chemically and photochemically quite stable. Hence, they may be good lead compounds for glycoconjugated photosensitizers. In addition, sugar moieties can easily be installed on the chlorin **2**. Fluorine at the *para*-position of a pentafluorophenyl group is known to be an excellent leaving group in nucleophilic substitution reaction with thiolate anions even at ambient temperature. Boyle et al. firstly developed a very efficient methodology for functionalization of porphyrin derivatives having pentafluorophenyl groups at the *meso*-positions by using thiol derivatives [22] or mixtures of Na_2S and electrophile [23]. By using this powerful methodology, *S*-glycosylated porphyrins have been synthesized by Drain et al. [24–26] and Boyle et al. [27]. Nevertheless, there has been no report on the photochemical properties of *S*-glycosylated chlorins. Here, we report the synthesis of *S*-glycosylated chlorins by using two methodologies, namely highly efficient 1,3-dipolar cycloaddition on porphyrin **1** and the substitution reaction on pentafluorophenyl groups with *S*-glycoside. The photophysical and chemical properties of the products, as well as their in vitro photocytotoxicity in HeLa cells, are also reported.

2. Materials and methods

2.1. Measurements

Elemental analyses were carried out using a Perkin–Elmer PE2400 Series II CHNS/O Analyzer at Nara Institute of Science and Technology. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded using JNM-AL400 (400 MHz, JEOL Ltd., Tokyo, Japan) and JNM-EC600 (600 MHz, JEOL Ltd., Tokyo, Japan) instruments. IR spectra were recorded on a FT-IR 8700 (Shimadzu Co., Kyoto, Japan). UV–vis spectra were recorded on a V-570 spectrophotometer (JASCO Co., Ltd., Tokyo, Japan). Steady-state fluorescence (FL) spectra were recorded on FP-6300 spectrofluorometer (JASCO Co., Ltd., Tokyo, Japan). Preparative gel permeation chromatography (GPC) was performed on LC-908 Recycling Preparative high-performance liquid chromatography (HPLC) system (Japan Analytical Industry Co., Ltd., Tokyo, Japan) equipped with two polystyrene columns (JAIGEL-2.5H and JAIGEL-2H), with $CHCl_3$ as an eluent. Preparative HPLC was carried out using octadecylsilyl-bound silica gel (Mightysil RP-18, 20 mm $\phi \times 250$ mm, KANTO CHEMICAL Co., Inc., Tokyo, Japan). Absorbance and fluorescence intensity of wells were determined with plate readers (Multiscan JX, Thermo Fisher Scientific Co., Yokohama, Japan and SPECTRA Fluor Plus, TECAN Group Ltd., Seestrasse, Switzerland, respectively). Bright field and fluorescence images of cells were taken by using a confocal laser scanning microscope (CLSM, Model LSM 510, Carl Zeiss, Jena, Germany).

2.2. Materials

2.2.1. General chemicals

All chemicals were of analytical grade. Tetraphenylporphyrin tetrasulfonic acid (TPPS) and 1,3-diphenylisobenzofuran (DPBF) were purchased from Sigma–Aldrich Japan (Chiba, Japan). 4′,6-Diamidino-2-phenylindole dihydrochloride hydrate (DAPI) was purchased from Molecular Probes (Eugene, OR). Porphyrin **1** [28], chlorin **2** [15], acetyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **a′** [29] and acetyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **b′** [29] were prepared according to the literature. The *S*-glucosylated porphyrin **1a′**, *S*-glucosylated porphyrin **1a** *S*-galactosylated porphyrin **1b′** and *S*-galactosylated porphyrin **1b** were synthesized by modifications (*vide infra*) of the literature procedures [25]. Stock solutions of photosensitizers were prepared by weighing the dried photosensitizers and dissolving them in dimethyl sulfoxide (DMSO, Wako Pure Chemical Industries, Osaka, Japan), and kept in freezer (−30 °C) until use.

2.2.2. *S*-glucosylated porphyrin (**1a′**)

Porphyrin **1** (64.9 mg, 66.6 μmol), *S*-glucoside **a′** (113 mg, 278 μmol) and diethylamine (3.0 mL) were dissolved in DMF (30 mL). The reaction mixture was stirred at room temperature for 24 h, diluted with $CHCl_3$ (30 mL) and washed with distilled water (30 mL × 5). The solution was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (silica gel, $CHCl_3$ to $CHCl_3$:AcOEt = 7:3) followed by preparative GPC to give *S*-glucosylated porphyrin **1a′** (116 mg, yield 74.0%) as a brownish purple solid. Anal. calcd. for $C_{100}H_{86}O_{36}N_4F_{16}S_4 + 2H_2O$: C, 50.30; H, 3.80; N, 2.35. Found: C, 50.24; H, 3.36; N, 2.19. ^{1}H NMR (600.07 MHz, $CDCl_3$, Si(CH_3)$_4$ = 0 ppm): δ (ppm) = 9.04 (8H, s, β-pyrrole*H*), 5.39 (4H, dd, ^3J = 9.3 Hz and 9.3 Hz, 3-Glc*H*), 5.26 (8H, m, 2,4-Glc*H*), 5.19 (4H, d, ^3J = 10.0 Hz, 1-Glc*H*), 4.33 (8H, dd, ^3J = 3.0 Hz, ^2J = 1.6 Hz, 6-Glc*H*), 3.92 (4H, dt, ^3J = 10.1 Hz, ^3J = 3.1 Hz, 5-Glc*H*), 2.24 (3H, s, CH_3), 2.11 (3H, s, CH_3), 2.10 (3H, s, CH_3), 2.09 (3H, s, CH_3), −2.85 (2H, brs, N*H*). ^{13}C NMR ($CDCl_3$, 100.40 MHz, $CDCl_3$ = 77 ppm): δ (ppm) = 170.37 (*C*=O), 169.91 (*C*=O), 169.22 (*C*=O), 169.14 (*C*=O), 147.76 (2,6-Ph*C*), 145.28 (3,5-Ph*C*, α-pyrrole*C*), 131.30 (β-pyrrole*C*), 121.95 (4-Ph*C*), 111.85 (1-Ph*C*), 104.24 (meso*C*), 84.43 (1-Glc*C*), 76.48 (5-Glc*C*), 73.96 (3-Glc*C*), 70.71 (2-Glc*C*), 68.18 (4-Glc*C*), 61.88 (6-Glc*C*), 20.75 (*C*H$_3$), 20.70 (*C*H$_3$). ^{19}F NMR (376 MHz, $CDCl_3$, C*F*$_3$CO$_2$H = −76.05 ppm): δ (ppm) = −132.13 (8F, dd, $^3J_{F-F}$ = 24 Hz, $^5J_{F-F}$ = 12 Hz, 3,5-Ph*F*), −136.43 (8F, dd, $^3J_{F-F}$ = 26 Hz, $^5J_{F-F}$ = 12 Hz, 2,6-Ph*F*). IR (KBr): ν (cm^{-1}) = 1755, 1470, 1369, 1227, 1061, 970.

2.2.3. *S*-glucosylated porphyrin (**1a**)

S-glucosylated porphyrin **1a′** (51.1 mg, 21.7 μmol) was dissolved in CH_2Cl_2 (10 mL) and MeOH (10 mL). Sodium methoxide (NaOMe) was added to adjust the pH to 9. This mixture was refluxed for 1 h at 45–50 °C, then neutralized with acetic acid. The solvent was removed, and the crude product was purified by preparative HPLC (CH_3CN:H_2O = 1:1). The collected fraction was desalted by dialysis using a cellulose ester membrane (molecular weight cut-off: 1000). Lyophilization of the desalted aqueous sample solution gave *S*-glucosylated porphyrin **1a** (24.9 mg, yield 68.8%) as a dark red powder. Anal. calcd. for $C_{68}H_{54}O_{20}N_4F_{16}S_4 + 6H_2O$: C, 45.69; H, 3.72; N, 3.13. Found: C, 45.57; H, 3.58; N, 3.14. ^{1}H NMR (600.07 MHz, CD_3OD, C*H*D$_2$OD = 3.30 ppm): δ (ppm) = 9.07 (8H, s, β-pyrrole*H*), 5.09 (4H, m, 1-Glc*H*), 3.92 (4H, m, 6-Glc*H*), 3.68 (4H, m, 6-Glc*H*), 3.44–3.22 (16H, m, 2,3,4,5-Glc*H*). ^{13}C NMR (100.40 MHz, CD_3OD, CD_3OD = 49.0 ppm): δ (ppm) = 149.70 (α-pyrrole*C*), 148.79 (α-pyrrole*C*), 147.18 (β-pyrrole*C*), 146.26 (β-pyrrole*C*), 125.00–135.00 (2,6-Ph*C* and 3,5-Ph*C*), 121.65 (4-Ph*C*), 115.02 (1-Ph*C*), 105.74 (meso*C*), 86.69 (1-Glc*C*), 82.73 (5-Glc*C*), 79.72 (3-Glc*C*), 75.96 (2-Glc*C*), 71.71 (4-Glc*C*), 63,10 (6-Glc*C*). ^{19}F NMR (376 MHz, CD_3OD, C*F*$_3$CO$_2$H = −76.05 ppm): δ (ppm) = −133.42 (8F, dd, $^3J_{F-F}$ = 25 Hz, $^5J_{F-F}$ = 12 Hz, 3,5-Ph*F*), −138.81 (8F, dd, $^3J_{F-F}$ = 24 Hz, $^5J_{F-F}$ = 12 Hz, 2,6-Ph*F*). IR (KBr): ν (cm^{-1}) = 3389, 1470, 1051, 968.