

Development of Novel Sugar Linked Photosensitizers for Photodynamic Therapy

Shigenobu Yano*

Division of Material Science, Graduate School of Human Culture, Nara Women's University,
Kitauoyanishimachi, Nara 630-8506, Japan

Sugar-linked porphyrin and chlorin compounds have been synthesized. Phototoxicity of these compounds against the HeLa cell line was also examined. For the porphyrin derivatives, the higher activity was observed for a derivative having four OH-protected sugar moieties. For the chlorin derivatives, OH-unprotected free-base derivatives were generally effective. Singlet oxygen producing ability were examined to evaluate the activity on photodynamic therapy of the compounds.

Key words: photodynamic therapy, singlet oxygen, porphyrin, chlorin, sugar

INTRODUCTION

Recently much attention has been focussed to photodynamic therapy (PDT) as clean and new modalities of selective treatments of cancer. This treatment, which results in the destruction of the cancer tissue, depends on the presence of molecular oxygen, and hence this is the example of the photodynamic effect. Porphyrin derivatives have been used as effective photosensitizers. However, the solubility into water, tumor selectivity, dark toxicity and a deep penetration of the light into tissue of these compounds are unsatisfied. Therefore, the develop-

ment of effective photosensitizers is highly desirable.

Since sugars have a number of OH groups and exists in important biological molecular recognition site, it can be expected that the introduction of sugar units to porphyrin derivatives increases water solubility and tissue selectivity. From this point of view, we have synthesized a series of new 5,10,15,20-tetrakis(*m*- or *p*-aldosylphenyl)-porphyrin (TPP) in addition to zinc(II) compounds, where D-glucose, D-galactose, D-xylose, and D-arabinose were used as a sugar unit. The reduction of TPP yielded corresponding 5,10,15,20-tetrakis(*m*- or *p*-aldosylphenyl)-chlorine (TPC) derivatives. Here we describe the combinatorial-like approach on order to evaluate the phototoxicity and singlet oxygen producing ability of the sixty-four derivatives of sugar-linked photosensitizers.

*To whom correspondence should be addressed.

E-mail: yano@cc.nara-wu.ac.jp

MATERIALS AND METHODS

Synthesis of the compounds. Porphyrin derivatives were synthesized from pyrrole and sugar-linked benzaldehyde by modified Lindsey's method [1,2]. Zinc-porphyrins were obtained by similar procedure in the presence of zinc acetate. Chlorin derivatives were prepared by the diimide reduction of corresponding porphyrins [3,4]. Overreduction was occurred, however, chloranil oxidation was effective to obtain the chlorin. Deprotection of these compounds by NaOMe in MeOH/CHCl₃ gave OH-

deprotected derivatives quantitatively. All new compounds gave expected spectrum properties and sufficient purity. The general structures of the compounds are shown in Figure 1. The detailed spectroscopic data of the compounds will be published elsewhere.

Singlet oxygen assay. The photosensitizing ability of these compounds, yielding singlet oxygen (¹O₂), was evaluated by degradation of diphenylisobenzofuran (DPBF) in DMSO according to the literature [1,2].

Phototoxicity against the HeLa cell. 10³ cancer cell incubated in the growth media (100 µl of MEM (Minimum Essential Medium) contains 10 % of Fetus Bovine Serum) at 37 °C overnight was incubated for 2 more hours in the presence of photosensitizer. The cell was washed with cold MEM, 200 µl of MEM was added, and irradiated for 8 minutes (light source: 500 W halogen lamp (λ > 500 nm for porphyrin derivatives and λ > 600 nm for chlorin derivatives), fluence 65 mW/cm²). Incubation was continued for 24 more hours and the number of surviving cells was analyzed by MTT assay.

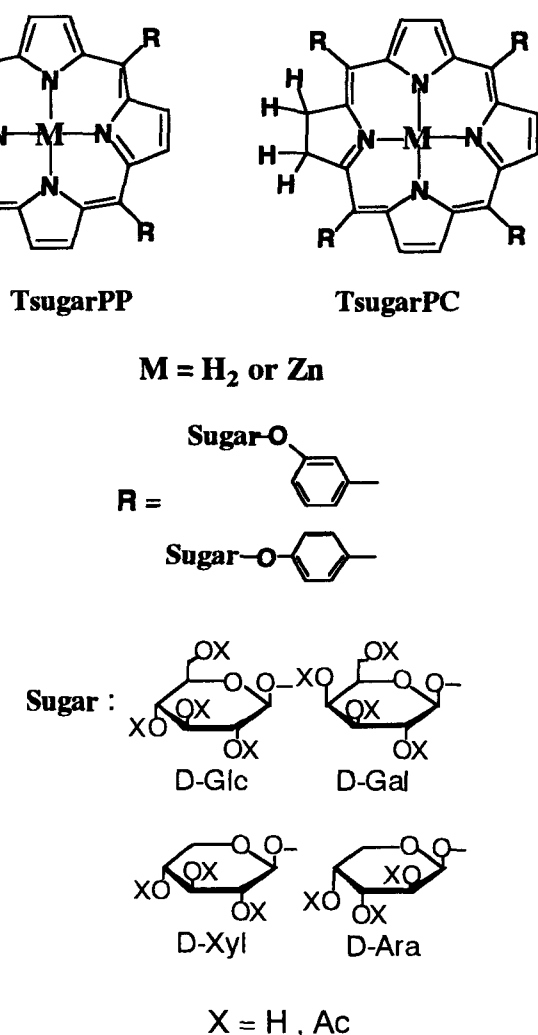


Figure 1. Structures of photosensitizers

RESULTS AND DISCUSSION

Synthesis. OH-protected sugar-linked porphyrin derivatives were successfully prepared by modified Lindsey's method from pyrrole and OH-protected sugar-linked benzaldehyde. Zinc ion promotes the reaction significantly by a template effect. Actually, *meta*-glucosilated porphyrin derivatives were not obtained in the absence of zinc acetate. Removal of zinc from zinc-porphyrin was performed by the treatment of 4M hydrochloric acid. Thus obtained OH-protected sugar-linked porphyrin was converted to corresponding chlorin by the diimide reduction [3,4]. The deprotection of the compounds were performed by sodium methoxide.

All chlorin compounds have larger molar

extinction coefficient on their Q_I band compared with corresponding porphyrin derivative Band I in their absorption spectra shifts to lower energy as expected with reduction of TPP (645 nm) to TPC (650 nm).

Table 1. Singlet Oxygen Producing Ability of Porphyrin Derivatives¹

Compounds	Relative Scale of ϕ ¹ O ₂
<i>p</i> -TacGlcPP	1.4
<i>p</i> -TGlcPP	1.3
Zn- <i>p</i> -TacGlcPP	3.6
<i>m</i> -TacGlcPP	1.3
<i>p</i> -TacGalPP	1.3
<i>p</i> -TacXylPP	1.5
<i>p</i> -TacAraPP	1.7
TPPS	1.0

¹Solvent: DMSO. Measurements were carried out under O₂ saturated condition at 27 °C. Light source: λ > 500 nm halogen lamp (250 W).

Table 2. Singlet Oxygen Producing Ability of Chlorin Derivatives¹

Compounds	Relative Scale of ϕ ¹ O ₂
<i>p</i> -TacGlcPC	1.1
<i>p</i> -TGlcPC	3.7
Zn- <i>p</i> -TacGlcPC	0.7
<i>m</i> -TacGlcPC	2.0
<i>p</i> -TacGalPC	0.3
<i>p</i> -TacXylPC	0.5
<i>p</i> -TacAraPC	1.6
TPPS	1.0

¹Solvent: DMSO. Measurements were carried out under O₂ saturated condition at 27 °C. Light source: λ > 600 nm halogen lamp (250 W).

Singlet Oxygen Producing Ability. These compounds produced singlet oxygen upon photoirradiation. This rate was measured by diphenylisobenzofuran (DPBF) in DMSO [1,2]. The observed rate constants (*k*_{obs}) are listed in Tables 1 and 2.

The photodynamic efficiency generating singlet oxygen for porphyrin derivatives is almost identical in all compounds. Retardation by sodium azide, which is a well-known singlet oxygen quencher, confirms that this reaction is accompanied with generation of singlet oxygen. Under the same experimental conditions, these sugar-linked porphyrins were almost as effective as hematoporphyrin (HP) and tetraphenylporphyrin tetrasulfonic acid (TPPS) which are known as photosensitizers that produce singlet oxygen efficiently. Zinc porphyrin derivatives show times as higher ability for ¹O₂ evolution than that of free-base porphyrin derivatives. This higher efficiency of zinc porphyrin may be due to the longer life-time of excited triplet state. The chlorin derivatives showed rather scatter trend for the singlet oxygen production.

Photocytotoxicity. Sugar-dependent significant differences in phototoxicity against the HeLa cells have been observed (Figure 2). All OH-deprotected compounds prepared increase solubility into water and have little or no dark cytotoxicity. Since the photodynamic efficiency in generating singlet oxygen is almost identical in all the porphyrin derivatives, the *in vitro* photocytotoxic results indicates that tetracarbohydrated porphyrins with OH-protected sugar by acetyl groups specifically increases the incorporation of the drug into the cell.

In contrast to the results from porphyrin derivatives, the higher activity was observed in OH-free compounds for tetraglycoconjugated chlorins. The detailed discussion about these results will be published elsewhere.

	<i>para</i> -Glycosylated Tetraphenylporphyrin				<i>para</i> -Glycosylated Zinc- Tetraphenylporphyrin				<i>para</i> -Glycosylated Tetraphenylchlorin				<i>para</i> -Glycosylated Zinc- Tetraphenylchlorin			
	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free
D-Glucose	1	2	5	6	3	4	7	8	11	12	15	16	19	20	23	24
D-Galactose	9	10	13	14	17	18	21	22	25	26	29	30	33	34	37	38
D-Xylose	25	26	29	30	33	34	37	38	41	42	45	46	49	50	53	54
D-Arabinose	25	26	29	30	33	34	37	38	41	42	45	46	49	50	53	54
	<i>meta</i> -Glycosylated Tetraphenylporphyrin				<i>meta</i> -Glycosylated Zinc- Tetraphenylporphyrin				<i>meta</i> -Glycosylated Tetraphenylchlorin				<i>meta</i> -Glycosylated Zinc- Tetraphenylchlorin			
	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free	OH-Protected	OH-Free
D-Glucose	33	34	37	38	35	36	39	40	43	44	47	48	51	52	55	56
D-Galactose	41	42	45	46	43	44	47	48	51	52	55	56	59	60	63	64
D-Xylose	49	50	53	54	51	52	55	56	59	60	63	64	67	68	71	72
D-Arabinose	57	58	61	62	59	60	63	64	67	68	71	72	75	76	79	80



Figure 2. Photocytotoxicity of compounds against the HeLa cell. 65: *m*-THPC (5,10,15,20-tetrakis(*m*-hydroxyphenyl)chlorin). [Photosensitizer]= 1.0×10^{-5} M Irradiation time was 8 min; the percentage of cell survival was determined by MTT assay after 24 h incubation. Light source: 500 W halogen lamp ($\lambda > 600$ nm), fluence 65 mW/cm² Cell survival: \doteq 10%; \doteq 20%; \doteq 50%; \geq 50% Cell survival of *m*-THPC \doteq 20%.

CONCLUSIONS

Here we show the synthesis and sugar-dependent phototoxicity of the sugar-linked porphyrin derivatives against the HeLa cell. These results are very important to develop the high functional photosensitizers in PDT

REFERENCES

1. Mikata, Y., Onchi, Y., Tabata, K., Ogura, S., Okura, I., Ono, H., and Yano, S. (1998) Sugar-dependent photocytotoxic property of tetra- and octa-glycoconjugated tetraphenylporphyrins *Tetrahedron Lett.* 39, 4505-4508.
2. Mikata, Y., Onchi, Y., Shibata, M., Kakuchi, T., Ono, H., Ogura, S., Okura, I., and Yano, S. (1998) Synthesis and phototoxic property of tetra- and octa-glycoconjugated tetraphenylchlorins. *Bioorg. Med. Chem. Lett.* 8, 3543-3548.
3. Whitlock, Jr., H. W., Hanauer, R., Oester, M. Y., and Bower, B. K. (1969) Diimide reduction of porphyrins *J. Am. Chem. Soc.* 91, 7485-7489
4. Bonnett, R., White, R. D., Winfield, U.-J., and Berenbaum, M. C. (1989) Hydroporphyrins of the meso-tetra(hydroxyphenyl)porphyrin series as tumour photosensitizers. *Biochem. J.* 261, 277-280