Chapter 3

Silica Gel–Supported P-, Ge-, and Sb-Porphyrins for Visible Light Inactivation of Bacteria

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Additional information is available at the end of the chapter

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Abstract

This chapter describes the photocatalysis action of (dihydroxo)tetraphenyl¬porphyrinato complexes of high valent P (V), Ge (IV), and Sb (V) (P(tpp), Ge(tpp), and Sb(tpp)). These chromophores were fixed onto silica gel (SiO2) through Coulombic forces and hydrogen bonding between axial hydroxo ligands and silanol groups to produce M(tpp)/SiO2 (M = P, Ge, and Sb) composites. M(tpp)/SiO2 were applied to the photo-inactivation of *Escherichia coli* and *Legionella pneumophila*. Moreover, M(tpp)/SiO2 was subjected to practical experiments for the photoinactivation of *L. pneumophila* naturally occurring in a cooling tower and a public fountain. It is noteworthy that 80 g of Sb(tpp)/SiO2 catalyst, containing 40 mg of Sb(tpp) maintained a concentration of Legionella species below 100 CFU/100 mL for 120 days in 13 m3 of water in a fountain under sunlight exposure. The photoinactivation proceeded through the liberation of M(tpp) from SiO2, adsorption of M(tpp) inside bacteria, and generation of reactive oxygen species, such as singlet oxygen, under visible light irradiation, thus resulting in bacteria apoptosis. Based on these results, we developed water-soluble porphyrins by modification of P and Sb porphyrin axial ligands to alkyloxo, alkylethylenedioxy, and alkylpyridinium groups. These water-soluble porphyrins were applied to the photodynamic inactivation of E. coli and *Saccharomyces cerevisiae*.

Keywords: high valent metal, P(V)-porphyrin, Ge(IV)-porphyrin, Sb(V)-porphyrin, *Escherichia coli*, *Legionella pneumophila*, *Saccharomyces cerevisiae*

1. Introduction

Photocatalysis has received much attention as an environmentally friendly process for degrading organic compounds and bacteria in contaminated water. It is well known that UV-light irradiation of TiO_2 generates a hydroxyl radical, which works as a strong oxidizing reagent

for various microorganisms in aqueous solutions [1]. In 1985, Matsunaga et al. reported that Pt-loaded TiO_2, under irradiation of >380 nm light, was capable of photoinactivation of various microorganisms, such as *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, *Escherichia coli*, and *Chlorella vulgaris* [2]. However, the UV light used for activation of TiO_2 is harmful to humans and only low amounts of the correct wavelengths are found in sunlight. Therefore, a photocatalyst that operates under irradiation from visible light emitted from the sun or a fluorescent lamp has been earnestly desired. Many researchers have focused much attention on porphyrins and metalloporphyrins, which can absorb visible light with high-absorption coefficients. Among the various metalloporphyrin chromophores, we selected axially dihydroxo-substituted tetraphenylporphyrin complexes (M(tpp)) of high valent metals, such as P(V), Ge(IV), and Sb(V) (**Figure 1**). To support M(tpp) on a carrier, we used silica gel (SiO_2), which has a high transparency to visible light and a strong binding force to porphyrins. Thus, M(tpp)/SiO_2 composites were prepared by adsorption of M(tpp) on SiO_2 and the composites operated as photocatalysts under visible light irradiation. Moreover, the porphyrins were subjected to water solubilization for medical field applications.

In this chapter, we will cover M(tpp)/SiO_2 and water-soluble porphyrins that were applied to the photosensitized inactivation of *E. coli* and *S. cerevisiae* in a reaction vessel and *Legionella pneumophila* in naturally occurring environments such as cooling tower and public fountains.

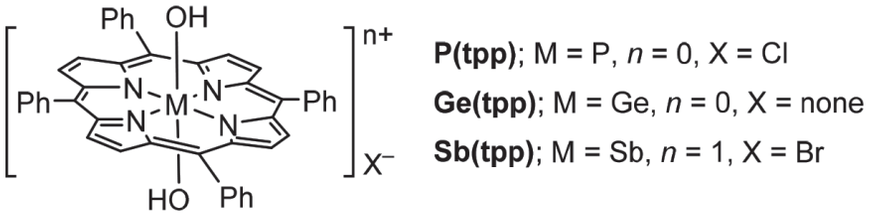

Figure 1. P(tpp), Ge(tpp), and Sb(tpp).

2. Preparation of the photocatalysts

2.1. Porphyrin chromophores

The porphyrin chromophores studied were (dihydroxo) tetraphenylporphyrinatophosphorus chloride (P(tpp)), (dihydroxo)tetraphenylporphyrinatogermanium (Ge(tpp)), and (dihydroxo)tetraphenylporphyrinatoantimony bromide (Sb(tpp)). Axial hydroxo ligands were bonded to the metals through stable covalent bonds. P(tpp) and Sb(tpp) are cationic complexes, whereas Ge(tpp) is a neutral complex. **Table 1** lists the physicochemical properties of the M(tpp)s, such as oxidation potentials ($E_{1/2}^{ox}$ vs. Ag/$AgNO_3$), reduction potentials ($E_{1/2}^{red}$), absorption maxima (λ_{max}), and molar absorption coefficients (ε). Since M(tpp) has absorptions at the Soret band around 420 nm and Q-bands at 550 nm with high ε, they can capture even weak light emitted from a fluorescent lamp and the sun. The fluorescence maxima (λ_F), quantum yields (Φ_F), and lifetimes (τ_F) were obtained from fluorescence spectra. Since Φ_F values were low, it was suggested that the efficiency of triplet state formation was relatively

M(tpp)	$E_{1/2}^{ox}$/V[a]	$E_{1/2}^{red}$/V[b]	λ_{max}/nm[c] (ε /10^4 M^{-1} cm^{-1})[d]		Fluorescence		Ref.
			Soret band	Q-band	λ_{max}/(E^{0-0}/eV)[e]	Φ_F (τ_F/ns)[f]	
P(tpp)	1.20[g]	−0.93[g]	424 (31.2)	554 (1.82)	607 (2.04)	0.0416 (5.4)	[3]
Ge(tpp)	0.95	−0.83	420 (77.6)	554 (2.19)	596 (2.08)	0.1500 (4.7)	[4, 5]
Sb(tpp)	1.17	−0.74	419 (41.6)	552 (3.09)	596 (2.08)	0.0518 (1.6)	[6, 7]

[a]Half peak of oxidation potential *vs* $Ag/AgNO_3$.
[b]Half peak of reduction potential *vs* $Ag/AgNO_3$.
[c]Absorption maxima of M(tpp).
[d]Molar absorption coefficients (ε) of M(tpp) in MeCN. The ε of P(tpp) was measured in MeOH.
[e]Fluorescence maxima. The values in parenthesis are excitation energy in eV.
[f]Fluorescence quantum yield (Φ_F) under excitation at the Q-band and fluorescence lifetimes (τ_F).
[g]Unpublished results.

Table 1. Properties of M(tpp).

high. High efficiency of triplet state formation is advantageous for energy transfer to $^3O_2$. The $E_{1/2}^{red}$ values of M(tpp)s were −0.74 to −0.93 V vs. $Ag/AgNO_3$, which were relatively positive compared with the divalent metal tetraphenylporphyrin complexes of Zn(II) (−1.31 V), Ni(II) (−1.18 V), and Pb(II) (−1.10 V) [8]. This fact shows that M(tpp)s (M = P, Ge, Sb) have powerful oxidation abilities under light irradiation. Moreover, the toxicity of M(tpp) is low. The lethal dose 50 (LD_{50}) of Sb(tpp) [9] and P(tpp) [10] was more than 2000 mg/kg. Therefore, M(tpp) can be safely used in living environmental fields.

2.2. Preparation of porphyrins/silica gel composites

To perform the photoreaction in an aqueous solution, less water-soluble M(tpp) was fixed onto porous SiO_2 to form M(tpp)/SiO_2 [11]. Silica gel powder (300 mesh, 40 μmϕ, BW300 Fuji Silysia, Japan) and silica gel beads (0.85–1.70 mmϕ, 306 m^2 g^{-1}, CARIACT Q–10, Fuji Silysia) were used. M(tpp) was fixed on SiO_2 through Coulombic forces and hydrogen bonding between axial hydroxo ligands and silanol groups. The general procedure for M(tpp)/SiO_2 preparation is described for the case of Sb(tpp)/SiO_2 as follows. SiO_2 (70 g) was added to a MeOH−toluene solution (1:4 v/v, 500 mL) containing Sb(tpp) (60 mg) and the mixture was allowed to stand for 18 h. MeOH was evaporated from the solution at 40°C under reduced pressure (**Figure 2**). The treated silica gel was isolated by filtration and then dried under reduced pressure at 40°C to give Sb(tpp)/SiO_2. The content of Sb(tpp) was 0.087 wt%.

2.3. Photocatalytic oxidation of organic compounds using M(tpp)/SiO_2 (M = Ge, Sb)

Since M(tpp)/SiO_2 (M = Ge, Sb) have high oxidation abilities, M(tpp)/SiO_2 was applied to the oxidation of cycloalkenes [12] and acetone [4] and the dechlorination of 4-chlorophenol [13]. The photocatalytic reactions were performed using the setup depicted in **Figure 3**, where the reactant was supplied by a continuous flow system. In a spiral-type apparatus (**Figure 3A**), the reactant solution is fed continuously from a holder to a spiral glass tube packed with the

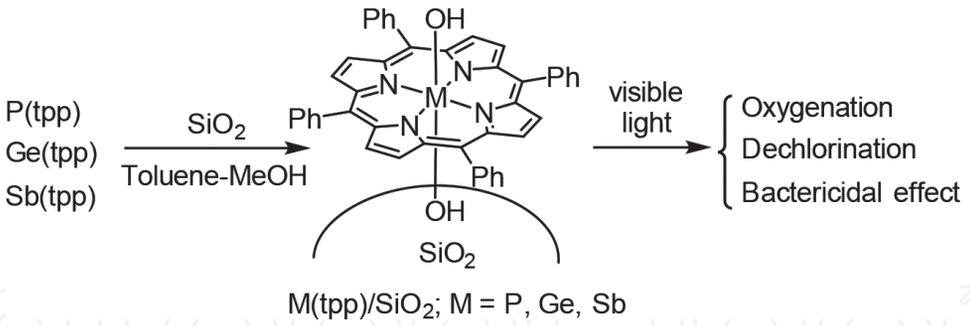

Figure 2. Silica gel-supported M(tpp) catalyst (M(tpp)/SiO_2).

photocatalyst. Sample irradiation was performed using a fluorescent lamp (22 W). The oval mirror-type apparatus (**Figure 3B**) consisted of a fluorescent lamp (18 W), an oval mirror, and a reactor (20 mmϕ × 500 mm; 150 mL) packed with the photocatalyst. The fluorescent lamp was set on one focus of the oval mirror, and the reactor was set at another focus. The visible light emitted from the fluorescent lamp was concentrated onto the reactor. The reactant solution was fed continuously from reservoirs into the reactors.

The photocatalytic oxidations of cycloalkenes using oxygen were performed in a spiral type reactor (**Figure 3A**). Irradiation was directed onto a spiral glass tube (4 mmΦ × 2.5 m) containing photocatalyst (6.0 g) to which CH_2Cl_2 solutions (150 mL) of cycloalkenes (0.1 M) were fed continuously from the reservoir [12]. To reduce silanol group effects, Sb(tpp)/SiO_2 was modified by capping with $(Me_3Si)_2NH$ to give Sb(tpp)/SiO_2^{TMS}. The photocatalytic oxidation of cyclohexene on Sb(tpp)/SiO_2^{TMS} in CH_2Cl_2 produced *cis*-1,2-epoxycyclohexane (28%), 1,2-cyclohexanediol (27%), and 2-cyclohexen-1-ol (38%) along with a small amount of 2-cyclohexene-1-one (7%) at 99.8% conversion after irradiation for 56 h (**Figure 4**). Similarly, the photocatalytic oxidation was applied to cyclooctene and 1-methyl-cyclohexene, which mainly resulted in diol formation.

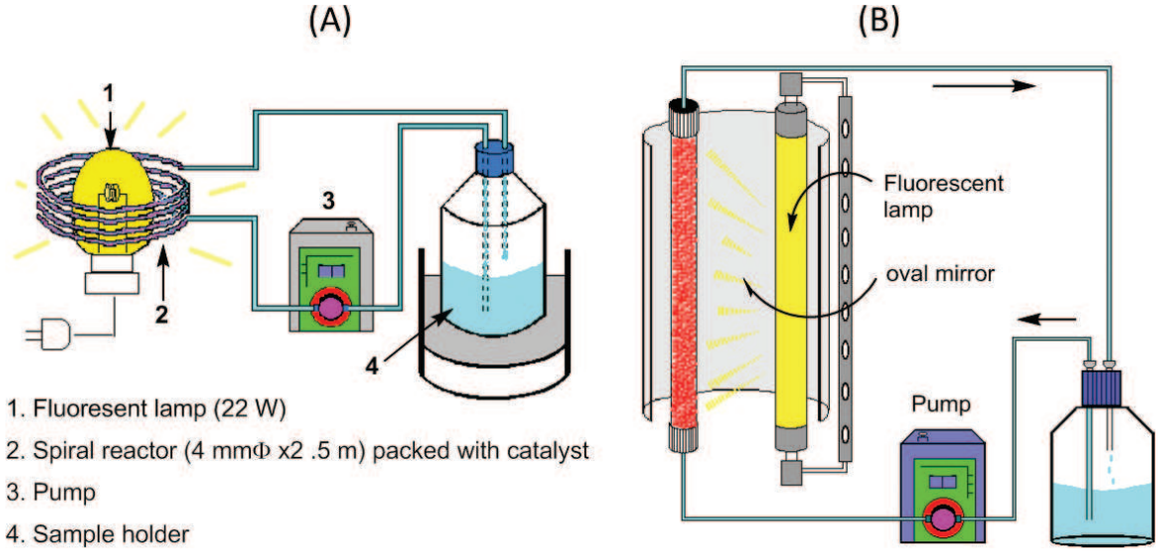

Figure 3. Setups for photocatalytic reactions: spiral type (A) and oval mirror type (B).

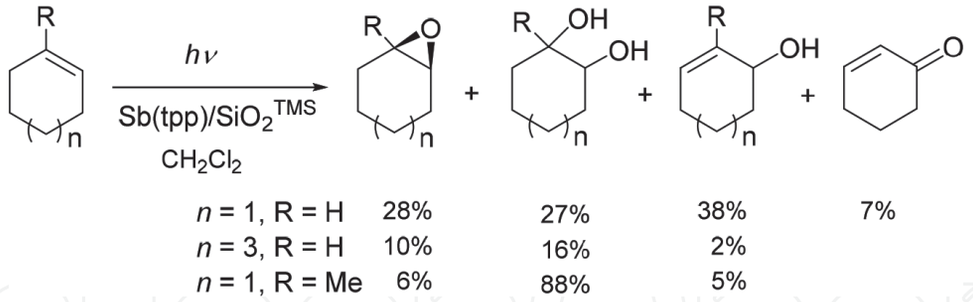

Figure 4. Photo-oxidation of cycloalkenes using $Sb(tpp)/SiO_2^{TMS}$.

Photo-oxidation of MeOH to HCHO was performed by $Ge(tpp)/SiO_2$ at room temperature using a spiral type reactor (**Figure 3A**). Irradiation with a fluorescent light was performed on a spiral glass tube (4 mmϕ × 2.5 m) packed with $Ge(tpp)/SiO_2$ (12 g). The reactant was fed continuously into the spiral glass tube from the reservoir that contained an aerated aqueous solution of MeOH (50 mM in 200 mL) [4]. HCHO (30.6 μM) was formed after 180 h with a turnover number (TON) of 3.0 (**Figure 5**). The isotope effect for the photo-oxidation of methanol was found to be 2.1 from the ratios of slopes in the time-conversion plots for CH_3OH and CD_3OH. Therefore, it was suggested that the oxidation occurs through hydrogen abstraction. The generation of a Ge-O• species was generated by excitation of Ge(tpp). Here, Ge(tpp) acts as an O-radical generator. The photo-oxidation by $Ge(tpp)/SiO_2$ was applied to toluene and ethylbenzene to produce alcohols and aldehydes/ketones. In the cases of cumene, methylcyclopentane, and methylcyclohexane, the corresponding alcohols were produced.

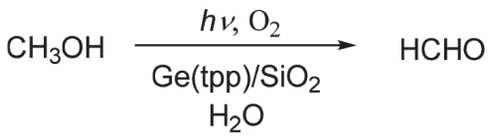

Figure 5. Photo-oxidation of methanol using $Ge(tpp)/SiO_2$.

Dechlorination of 4-chlorophenol (4-CP) using $Sb(tpp)/SiO_2$ was performed using the oval mirror-type apparatus (**Figure 3B**) [13]. Here, $Fe(NO_3)_3$ was used instead of O_2 as the electron acceptor for the dechlorination of 4-CP, since the oxidation potential of 4-CP was relatively high. $Sb(tpp)/SiO_2$ (0.087 wt% Sb(tpp)) was loaded into the oval mirror reactor. Before irradiation, the aqueous solution of 4-CP (initial concentration was 493 μM) was fed for 3 h under dark conditions, and the initial concentration of 4-CP decreased to 400 mM, probably due to the adsorption of 4-CP on the SiO_2. Upon irradiation with the fluorescent lamp for 72 h, the concentration of 4-CP decreased from 400 to 6 μM along with the formation of Cl^- (233 μM) and 1,4-benzoquinone (205 μM). Fe^{2+} (811 μM) was produced as a consequence of the reduction of Fe^{3+} (**Figure 6**). Electron transfer from the excited triplet state of $Sb(tpp)/SiO_2$ to Fe^{3+} was responsible for the photodechlorination initiation, since Rehm-Weller equation calculated that the free energy change (ΔG) for the electron transfer from the excited triplet state of Sb(tpp) (E^{0-0} = 1.63 eV) to Fe^{3+} ion ($E_{1/2}^{red}$ = −0.31 V vs $Ag/AgNO_3$) would be −0.15 V: $\Delta G = E_{1/2}^{ox} - E_{1/2}^{red} - E^{0-0}$, where $E_{1/2}^{ox}$ (Sb(tpp)) = 1.17 V vs $Ag/AgNO_3$. The resulting reactive

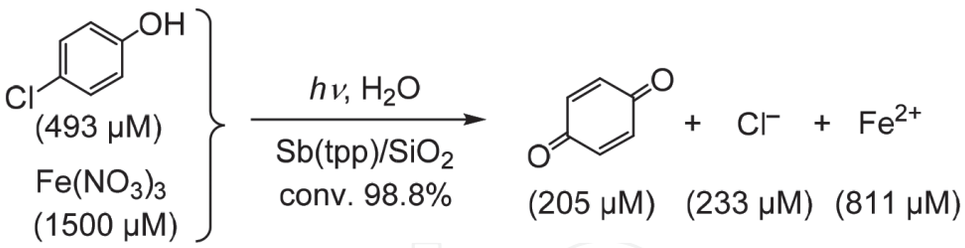

Figure 6. Dechlorination of 4-chlorophenol (4-CP) using Sb(tpp)/SiO_2 under irradiation from a fluorescent lamp.

dicationic Sb(tpp)/SiO_2 undergoes the hole transfer to 4-CP adsorbed on SiO_2 and solved in the aqueous solution. The resulting 4-CP cation radical allows for the nucleophilic addition of H_2O. The substitution of Cl with OH via the hydroxy adduct followed by the oxidation by Fe^{3+} gives 1,4-benzoquinone.

3. Photoinactivation of *E. coli* by M(tpp)/SiO_2 (M = P, Sb)

Our first experiment for photoinactivation using Sb(tpp)/SiO_2 was reported in 2003 against *E. coli* [10]. Photoinactivation of *E. coli* was enacted as follows: *E. coli* k-12 (IFO3335) was cultured aerobically at 30°C for 8 h in a basal medium (pH 6.5) consisting of 1% bactotriptone, 0.5% yeast extract, and 1% NaCl. After centrifugation of the cultured broth at 8500 g for 10 min, the harvested cells were washed with physiological saline and then resuspended in the saline. Phosphate buffer (9.0 mL, 100 mM, pH 7.0), the cell suspension of *E. coli* (1.0 mL, ca. 10^4 cells mL^{-1}), and Sb(tpp)/SiO_2 (10 mg) were introduced into an L-type glass tube (length 180 mm, diameter 15 mm) that was set on a reciprocal shaker in the apparatus shown in

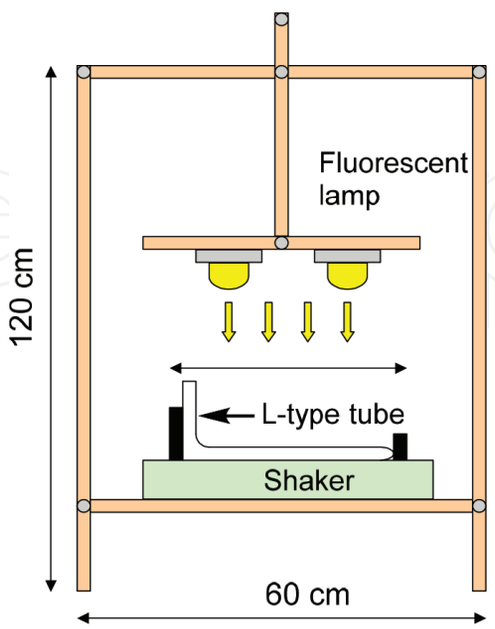

Figure 7. Apparatus used for photoinactivation of bacteria experiments.

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Figure 7. The glass tube was shaken at 160 rpm and irradiated for 2 h using a fluorescent lamp (Panasonic FL-15ECW, Japan, λ = 400–723 nm, maximum intensity at 545 nm, 10.5 W cm^{-2}) set above a reciprocal shaker. Aliquots (0.1 mL) were taken from the reaction mixture at 20 min intervals and plated on agar plates in triplicate. The colonies that appeared after incubation for 24 h at 30°C were counted. The amount of living cells (B in cell mL^{-1}) was defined as the average number of the colonies of *E. coli* in the triplicate plates.

Figure 8A shows the time courses of survival ratio (100 B/B_0) of *E. coli*, where B_0 is the initial amount of *E. coli*. Upon irradiation for 60 min, the concentration of *E. coli* decreased from the initial concentration (6.4 × 10^3 cells mL^{-1}) to about 40 cells mL^{-1}. Its survival ratio was 0.6%. Thus, Sb(tpp)/SiO_2 demonstrated bactericidal activity under visible light irradiation. Control experiments, irradiation in the absence of the photocatalyst and photocatalyst without irradiation, were also performed. Each control run maintained the B_0 of *E. coli*, showing that the Sb(tpp)/SiO_2 has bactericidal activity only with light activation. Incident light was absorbed exclusively by Sb(tpp)/SiO_2. However, the excited singlet state of Sb(tpp)/SiO_2 was too short-lived to contact directly with the bacteria. Therefore, it was suggested that the excited triplet state of Sb(tpp)/SiO_2 (triplet energy = 1.63 eV) underwent energy transfer to molecular oxygen (triplet energy = 0.98 eV) to generate singlet oxygen ($^1O_2$). Thus, we found that Sb(tpp)/SiO_2 could generate $^1O_2$ in aqueous solutions to sterilize *E. coli* cells using visible light irradiation.

Similar photoinactivation studies of *E. coli* were performed using P(tpp)/SiO_2 (10 mg) in phosphate buffer (10 mL) containing *E. coli* (10^4 cells) [10]. The survival plots are shown in **Figure 8B**. Under a nitrogen atmosphere, photoinactivation using P(tpp)/SiO_2 did not occur. We postulated that photoinactivation obeyed a Michaelis-Menten type mechanism, which involves an interaction between bacteria and P(tpp)/SiO_2 in the ground state. Plots of B_0–B

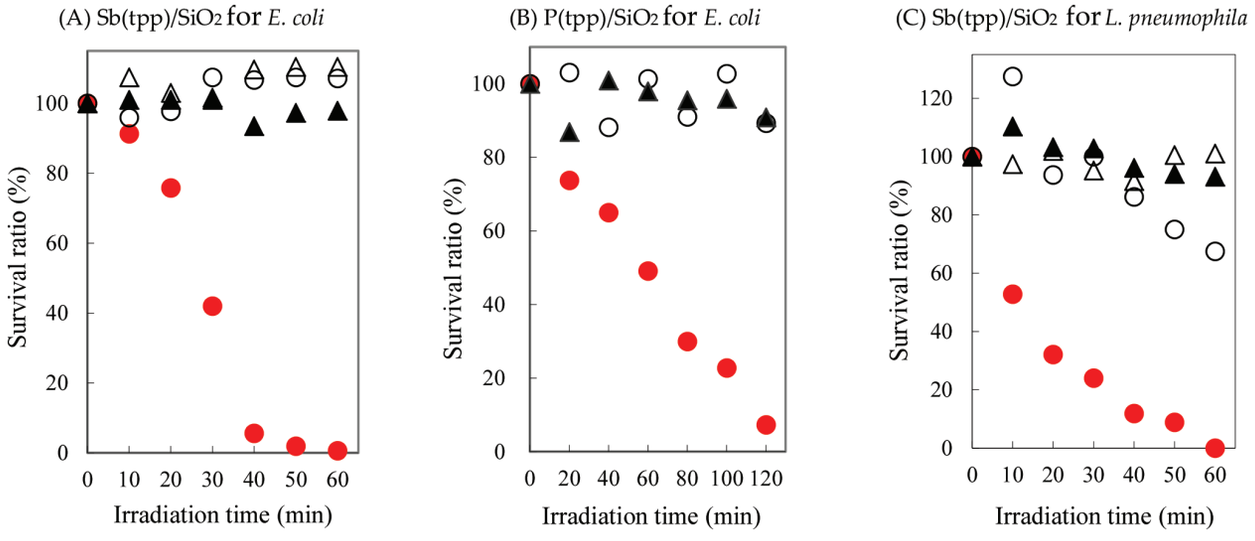

Figure 8. Photoinactivation of *E. coli* by Sb(tpp)/SiO_2 (A) and P(tpp)/SiO_2 (B) and *L. pneumophila* by Sb(tpp)/SiO_2 (C) under visible light irradiation in the presence of M(tpp)/SiO_2 (●), dark conditions in the presence of M(tpp)/SiO_2 (▲), visible light irradiation in the absence of M(tpp)/SiO_2 (○), and dark in the absence of M(tpp)/SiO_2 (△). Reaction conditions: initial concentration of bacteria = 1.0 × 10^4 cell mL^{-1} for *E. coli* and 6.4 × 10^3 cells mL^{-1} for *L. pneumophila*, M(tpp)/SiO_2 = 10 mg in phosphate buffer solution (10 mL) for *E. coli* and water (10 mL) for *L. pneumophila* under irradiation of fluorescent lamps (light intensity = 21 W cm^{-2}).

values against irradiation time gave a linear correlation. From the slopes of the plots, the rate for photoinactivation was determined to be proportional to the amounts of P(tpp)/SiO_2 and light intensity. Thus, adsorption of *E. coli* on P(tpp)/SiO_2 was important for efficient photoinactivation using P(tpp)/SiO_2 under visible light irradiation.

4. Photoinactivation of *Legionella* species in naturally occurring environments

L. pneumophila has become one of the most aggressive pathogens since the first outbreak of Legionnaires' disease in Philadelphia in 1976 [14]. The natural habitats for *L. pneumophila* include a wide range of aquatic bodies, such as lakes, streams, and artificially constructed aquatic reservoirs (hot springs, fountains, and cooling towers). Large outbreaks of Legionnaires' disease have been reported in Portugal, the Netherlands, and Spain [15–17]. On the other hand, photocatalytic treatments have received much attention as an environmentally friendly process to inactivate bacteria in contaminated water. In 2002-2003, our group carried out the photoinactivation of *Legionella* species occurring in bacteria's natural habitats, such as in cooling towers and public fountains using a use of Sb(tpp)/SiO_2 [18].

4.1. Photoinactivation of *L. pneumophila*

Initially, the photoinactivation of *L. pneumophila* was examined with Sb(tpp)/SiO_2 using L-type glass tubes in the apparatus shown in **Figure 7** in a similar manner to the method described for *E. coli* [10]. A phosphate buffer (0.1 M, pH 7.0, 10 mL) containing a cell suspension of *L. pneumophila* (6.4×10^5 CFU/100 mL), and Sb(tpp)/SiO_2 (10 mg) was introduced into the L-type glass tube (length 18 cm, diameter 1.5 cm) and irradiated with a fluorescent lamp [18]. Aliquots (0.1 mL) of the reaction mixture were directly plated on a selective medium for *Legionella* species, i.e., WYOα agar medium (Eiken Chemicals Co., Ltd, Japan) consisting of glycine (3 g), vancomycin (5 mg), polymixin B (10^6 IU), and amphotericin B (80 mg). The colonies of *L. pneumophila* appeared after incubation for 7 days at 36°C. Wet, smooth, and bluish-white colonies were counted on triplicate plates. The cell concentration of *L. pneumophila* was represented in colony formation units in 100 mL of the aqueous solution (CFU/100 mL).

Figure 8C shows the time courses for *Legionella* species survival. Upon irradiation for 60 min in the presence of the Sb(tpp)/SiO_2, the concentration of *Legionella* species apparently decreased from the initial concentration (6.4×10^5 CFU) to 4×10^3 CFU. Its survival ratio was 0.6%. In the control experiments, irradiation in the absence of Sb(tpp)/SiO_2, in the presence of Sb(tpp)/SiO_2 without irradiation, and in the absence of Sb(tpp)/SiO_2 and irradiation, each runs maintained the initial concentration of *Legionella* species. Thus, Sb(tpp)/SiO_2 was confirmed to have the photocatalytic activity that could sterilize *Legionella* species.

4.2. Practical experiments in a cooling tower

The bactericidal experiment was performed in a cooling tower (**Figure 9A**) that was located in a building in Miyazaki city [18]. A cylindrical apparatus (200 mmϕ × 500 mm, **Figure 9B**)

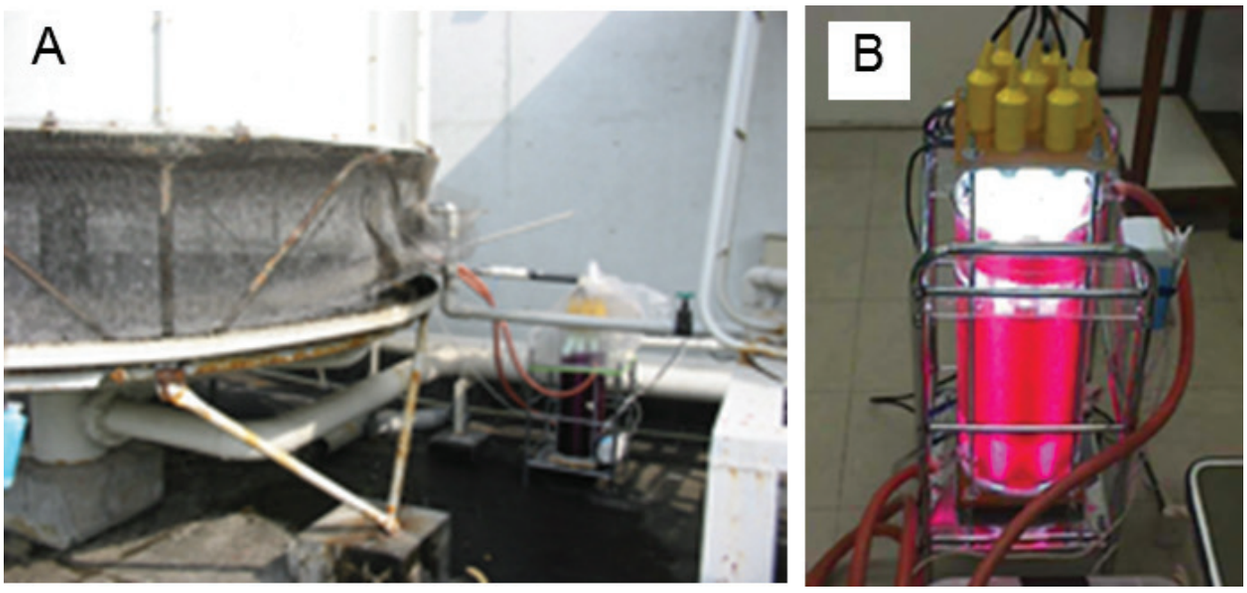

Figure 9. Practical experiment in a cooling tower: the cylindrical apparatus (B) setup in the cooling tower (A).

that consisted of seven fluorescent lamps (18 W, 43 mmϕ × 50 cm) and the Sb(tpp)/SiO_2 catalyst (4.0 kg, 0.05 wt% Sb(tpp)) was used. Water in the holder (800 L) of the cooling tower was pumped into a cylindrical vessel at a rate of 28 L min^{-1}, and then, the treated water was returned to the holder. The average retention time was calculated to be 26 s. In the cylindrical vessel, the Sb(tpp)/SiO_2 catalyst was irradiated by visible light emitted from the fluorescent lamps at ambient temperature. Sampling of the water was carried out at outlet at 3–7 day intervals. At the same time, the atmospheric temperatures were recorded as the average value of the highest temperature of Miyazaki city during the sampling day and 2 days prior. The sample water (1.0 L) was filtrated through a membrane filter (0.45 μm, HA, Millipore) under reduced pressure into the vessel (100 mL) containing the microbes adhering to the membrane filter, an aqueous solution (5 mL) was added, and the vessel was shaken vigorously. Saturated aqueous KCl (5 mL, pH 2.2) containing 0.2 M HCl was added, and the vessel was shaken vigorously. After standing for exactly 20 min at room temperature, the prepared solution was ready for plating as described in Section 4.1. The amounts of *Legionella* species were determined by the colony counting method.

Under the conditions without any bactericidal treatments, *Legionella* species occurred in a range from 20 to 139 CFU in the holder of the cooling tower, as shown in **Figure 10**. After the bactericidal apparatus was active, the concentrations of *Legionella* species decreased to levels below the detection limit. This was maintained until the irradiation treatment ceased. Seven days after the irradiation ceased, detectable amounts of *Legionella* species reappeared. Thus, the bactericidal effects of Sb(tpp)/SiO_2 were practically confirmed in this cooling tower experiment.

4.3. Practical experiments in water fountain

Practical experiments were performed in a public fountain of Miyazaki city (**Figure 11A**) that was filled with 13 m^3 of water [18]. Photoinactivation of the fountain was examined using a

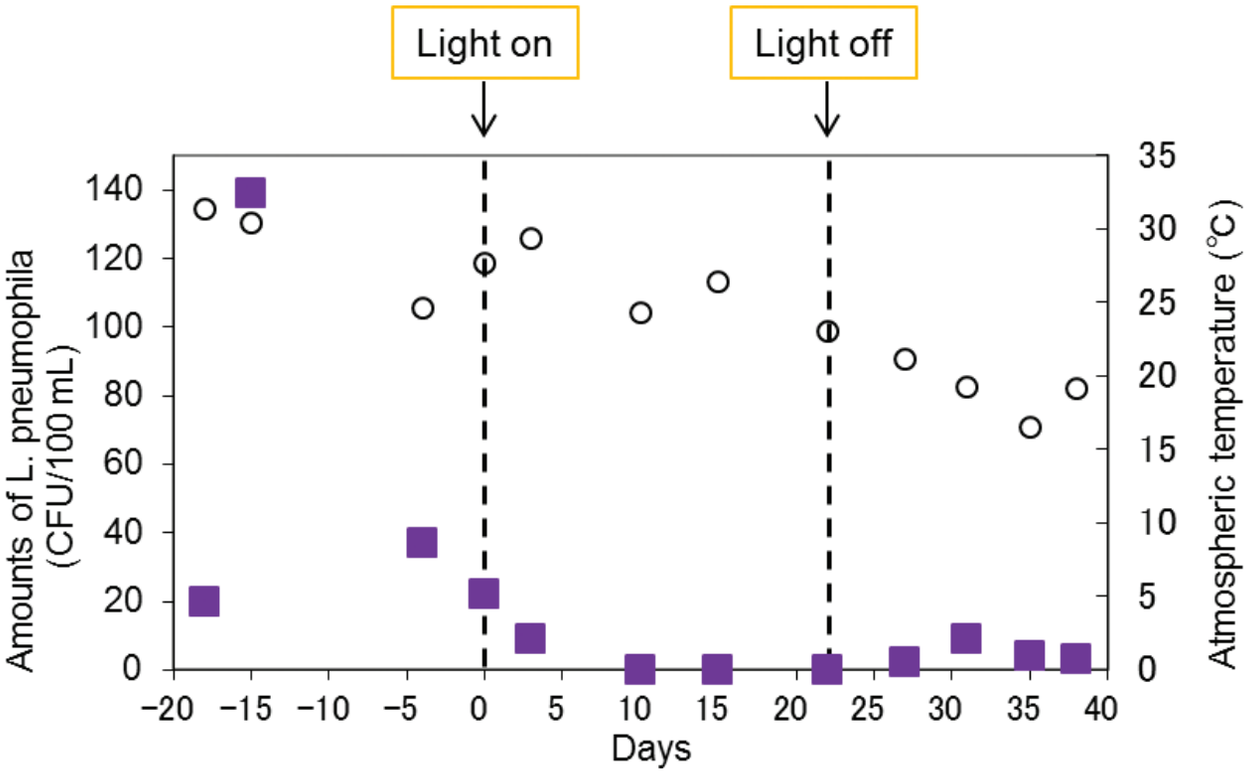

Figure 10. Time-course plots of the amounts of *Legionella* species (■) in the cooling tower along with the atmospheric temperature (○). The cylindrical photo-bactericidal apparatus was operated from October 1 to October 21, 2002. Conditions: catalyst, Sb(tpp)/SiO_2 (4 kg); water, ca. 800 L; flow rate, 28 L min^{-1}; and average retention time, 26 s.

leaf-type of photoinactivation apparatus (200 mmϕ × 50 mm, **Figure 11B**) containing the Sb(tpp)/SiO_2 catalyst (80 g, 0.05 wt% Sb(tpp)), which operated under sunlight irradiation. The determination of viable cell numbers of *Legionella* species was carried out in the manner described in Section 4.2. After the leaf-type apparatus had been installed into the fountain, the concentrations of *Legionella* species in the fountain were continuously kept below 30 CFU under sunlight irradiation (**Figure 12**). After the leaf-type apparatus was removed, the concentrations of *Legionella* species gradually increased to reach 100 CFU, which is the environmental quality standard, within 42 days after the removal.

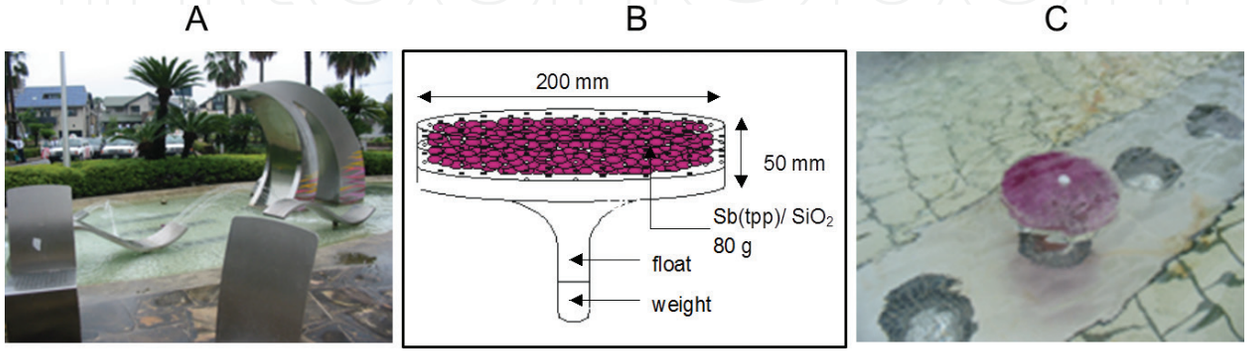

Figure 11. Practical experiments in a public fountain: The leaf-type apparatus (B) was set in the fountain (A). (C) Picture indicates the leaf-type apparatus set in the pool of the fountain.

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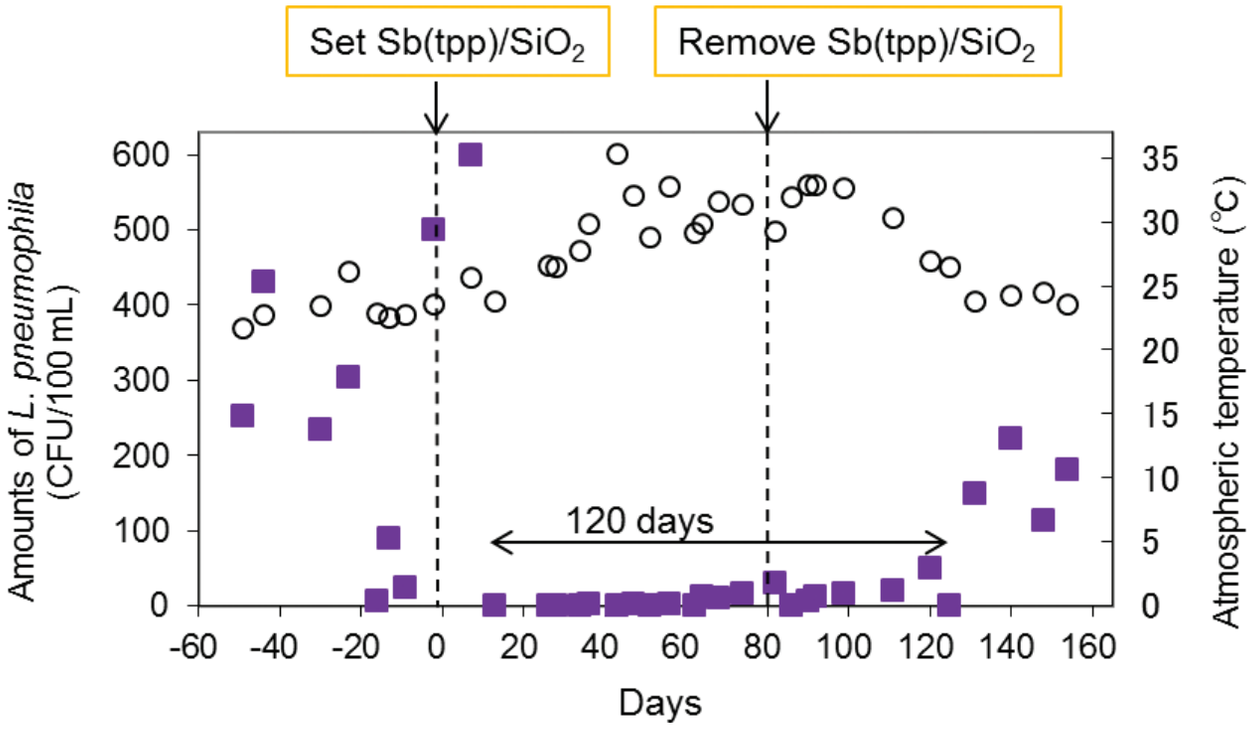

Figure 12. Time-conversion plots of the amount of *Legionella* species (■) in public fountain photoinactivation. The experiment was performed using a leaf-type photo-bactericidal apparatus containing Sb(tpp)/SiO_2 (80 g) in a fountain that contained 13 m^3 of water between April 9 to October 29, 2003. The leaf-type apparatus was installed into the fountain on May 28, 2003 and was removed from the fountain on August 22, 2003. Atmospheric temperature (○) was recorded as the average of the highest temperature of Miyazaki city for three days before the sampling day.

As a result of the practical experiments, it is noteworthy that 80 g of Sb(tpp)/SiO_2 catalyst, which contained 40 mg of Sb(tpp), could maintain the concentration of *Legionella* species in 13 m^3 of water below 100 CFU for 120 days.

4.4. Mechanism for photoinactivation using M(tpp)/SiO_2

Elemental analyses of the catalysts before and after use in the fountain were performed with ICP. Before use, the Sb content in Sb(tpp)/SiO_2 was measured to be 80 ppm, which was in good agreement with the Sb content (72 ppm) calculated for the 0.05 wt% of Sb(tpp) content in the catalyst. After 3 months of use in the fountain, the Sb content decreased from 80 to 17 ppm. On the other hand, Na, Mg, Al, and Ca largely increased, resulting in ion-absorption on SiO_2. Moreover, Sb(tpp)/SiO_2 catalyst used in the fountain was analyzed by a confocal laser scanning microscopy (CLSM). It was found that the fluorescence coming from the surface of the catalyst keep the similar shapes to the original catalyst, but the intensity was weaker compared with the original spectra of Sb(tpp)/SiO_2. On the other hand, the fluorescence from the inside of the catalyst maintained the original intensity. Therefore, it is suggested that Sb(tpp) was eliminated from the surface of the catalyst. Irradiation of fluorescent light on the Sb(tpp)/SiO_2 catalyst in deionized water did not sufficiently account for the spectral change and decrease in total Sb. Therefore, that the cationic Sb(tpp) chromophore was exchanged with alkali metal ions in the bulk water on the surface of the catalyst under irradiation is strongly suggested.

A similar phenomena were observed in the case of P(tpp)/SiO_2 [10]. Moreover, when Sb(tpp) was tightly fixed on SiO_2 through covalent bonds, no photoinactivation occurred [19].

Therefore, the liberation of the Sb(tpp) chromophore from SiO_2 is necessary for photoinactivation, as shown in **Figure 13**. Sb(tpp) can dissolve slightly in water (the water solubility (C_W) of Sb(tpp) is low (0.08 mM)). The liberated Sb(tpp) might be adsorbed by the bacteria and induce apoptosis under visible light irradiation. After the photoinactivation, Sb(tpp) might separate from the SiO_2 and move to the pool of the fountain. This may explain the loss of Sb(tpp) from Sb(tpp)/SiO_2.

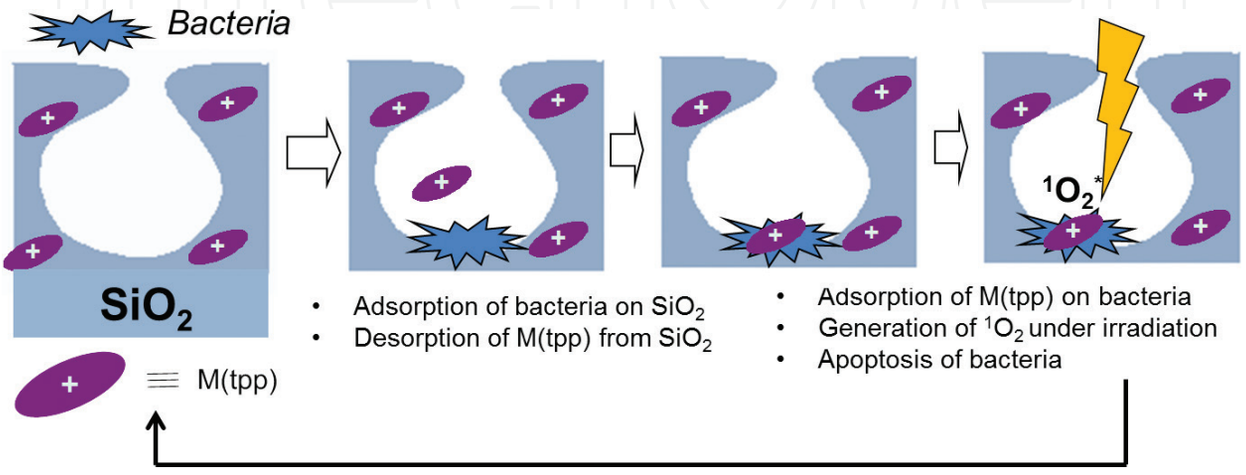

Figure 13. Possible mechanism for photoinactivation of bacteria by M(tpp)/SiO_2.

5. Photosensitized inactivation using water-soluble porphyrins

As mentioned in the previous section, it was found that Sb(tpp) dissolved in water was responsible for the photoinactivation of bacteria. Therefore, in our next study, we intended to inactivate bacteria using water-soluble porphyrins. Water-soluble porphyrins have received much attention in connection with photoinactivation [20] and photodynamic therapy (PDT) [21–24] ever since the first report on photoinactivation of *E. coli* by water-soluble *meso*-substituted cationic porphyrins appeared in 1996 [25]. For the biological application of porphyrins, water solubility is an important characteristic in the handling of porphyrins in an aqueous solution. We modified the axial ligands of P and Sb porphyrins by installing alkyloxo (**1**) [26], alkylethylenedioxy (**2**) [27, 28], and alkylpyridinium groups (**3**) [20, 29] (**Figure 14**). The water solubility (C_W in mM) is shown in **Table 2**. The quantum yields for the formation of $^1O_2$ were determined to be 0.65 for **1b**, 0.53 for **1d** [26], 0.62 for **2b**, 0.69 for **2c**, 0.73 for **2d** [27], 0.88 for **3g**, and 0.87 for **3c** [29].

We show the results of photoinactivation of *S. cerevisiae* and *E. coli* using water-soluble P and Sb porphyrins (**1**–**3**). Photoinactivation was enacted using the apparatus shown in **Figure 7**. The porphyrin solution, a bacteria suspension, and buffer solution (or water) were introduced into L-type glass tubes, resulting in a solution (10 mL) containing bacteria cells

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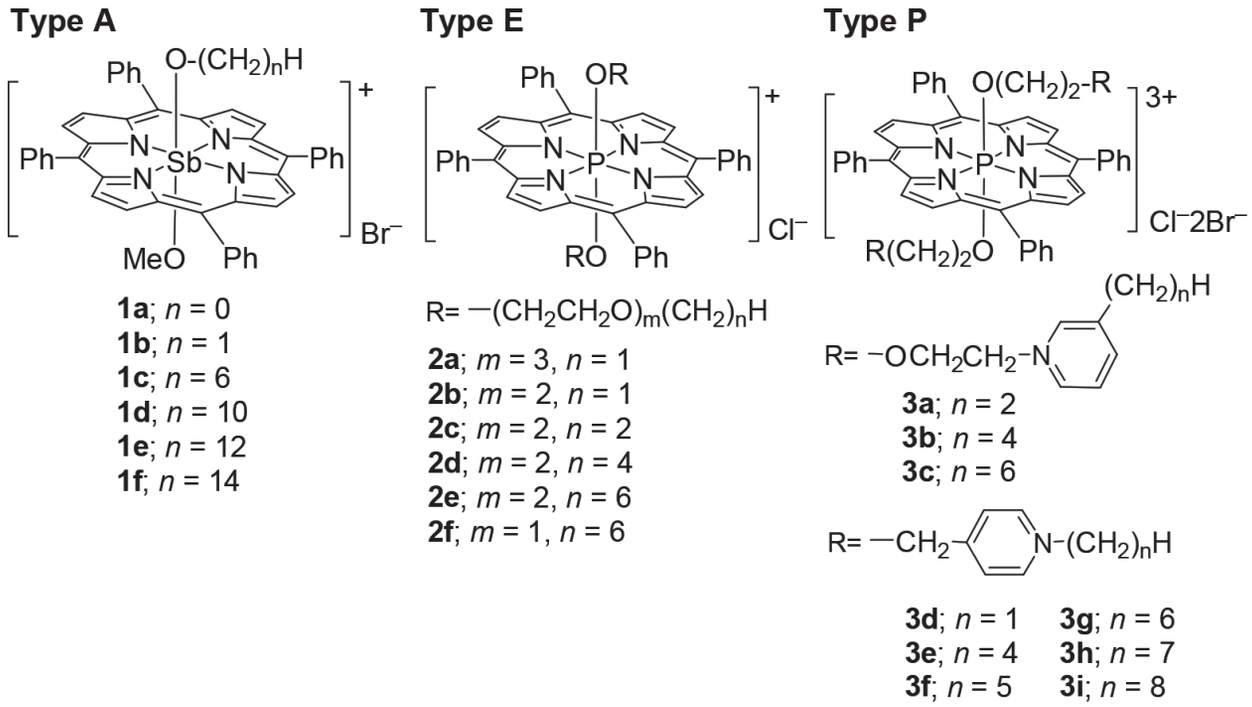

Figure 14. Water-soluble porphyrins (**1–3**).

(ca. 1×10^4 cell mL^{-1}) and compounds **1–3** (5–2000 nM). The solution was irradiated using a fluorescent lamp (λ_{max} = 545 nm) under aerobic conditions on a reciprocal shaker. The amounts of bacteria (cell mL^{-1}) at every irradiation time were determined by the colony counting method. Under dark conditions, the bacterial counts were maintained at the initial levels. Thus, it was confirmed that all porphyrins (**1–3**) have no bactericidal activity under dark conditions at all.

From the plots of the survival ratios against irradiation time, the bactericidal activity of porphyrins was evaluated by the half life ($T_{1/2}$ in min), which was the time required for the initial bacteria concentration to be halved. Moreover, the minimum concentrations ([*P*]) of porphyrins were adjusted until $T_{1/2}$ was found in the range of 0–120 min. The [*P*] values and $T_{1/2}$ are shown in **Table 2**. As [*P*] values and $T_{1/2}$ are smaller, the bactericidal activity of porphyrins is higher. The most active sensitizer for *S. cerevisiae* was **2e** and **2f**, whose [*P*] value was 5.0 nM. However, **2e** and **2f** were ineffective for *E. coli*. Tricationic complexes **3a–3c** were effective for *E. coli.* The bioaffinity of the porphyrins can be related to the structure of the bacterial cell wall. In the case of *S. cerevisiae*, whose cell wall consisted of hydrophobic peptidoglycan, water-soluble porphyrins (**2e–2f**) having hydrophobic character had the highest bioaffinity. On the other hand, polycationic porphyrins (**3**) had the highest bioaffinity toward gram-negative *E. coli*, whose cell wall consisted of phospholipids, lipopolysaccharides, lipoteichoic acids, and lipoproteins [30]. Moreover, it was found that **3** interacted strongly with human serum albumin (HSA) [31], and the addition of HSA was effective for inducing photoinactivation of *S. cerevisiae* using **3** [29]. Recently, reviews on photoinactivation by water-soluble porphyrins have been published by Almeida at al. [20] and our group [32].

Entry	*n*	*m*	C_W/mM	*S. cerevisiae*[b]		*E. coli*	
				[*P*]/nM	$T_{1/2}$/min	[*P*]/nM	$T_{1/2}$/min
1a	0		0.10	50	380	–	–
1b	1		0.13	50	192	–	–
1c	6		1.09	50	14	–	–
1d	10		2.10	40	22	–	–
1e	12		2.21	50	17	–	–
1f	14		2.40	50	21	–	–
2a	1	3	17.4	500	23	–	–
2b	1	2	13.9	300	81	–	–
2c	2	2	13.0	200	31	–	–
2d	4	2	5.38	50	55	–	–
2e	6	2	2.07	5	64	–	–
2f	6	1	1.11	5	69	–	–
3a	2		>120	*50	*32	250[c]	32[c]
3b	4		112	*50	*36	250[c]	53[c]
3c	6		63.6	50	44	250[c]	120[c]
3d	1		3.35	50	20	2000	66
3e	4		6.10	*30	*85	2000	27
3f	5		3.80	–	–	500	29
3g	6		5.84	*20	*72	500	31
3h	7		6.00	–	–	400	24
3i	8		3.80	–	–	500	63

[a]Water solubility of porphyrins in mM.

[b]Experiment with * was performed in the presence of human serum albumin (HSA, 400 nM).

[c]Unpublished results.

Table 2. Photoinactivation of *S. cerevisiae* and *E. coli* by water-soluble porphyrins (**1**, **2**, and **3**).

6. Conclusion and perspectives

Since aqueous solutions are more transparent for visible light than ultraviolet, visible light photocatalysts work best for the photocatalytic reactions in aqueous solution. Moreover, visible light photocatalysts take advantage of the photocatalytic reactions under sunlight irradiation, since sunlight consists of 52% visible, 42% infrared, 6% UV-A, and 0.5% UV-B light. We showed two methods to photoinactivate bacteria: one method is the dispersion of M(tpp)/SiO_2 (M = P, Ge, and Sb) in water, which is applicable to open system in naturally occurring environments; the other method is the water solubilization of M-porphyrins (M = P and Sb), which can be used in a closed system. M(tpp) and M-porphyrins can interact with bacteria through adsorption onto cell walls and absorption into the cells. Under irradiation, reactive

species, such as $^1O_2$, is generated by energy transfer from the porphyrins to O_2 molecules on the cell walls and inside the cells.

Thus, porphyrins are useful chromophores for catalysis and sensitization in a biological application. The application of water-soluble M-porphyrins to PDT is currently underway in our laboratories.

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