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Visible light-assisted sterilization activity of water-soluble antimonyporphyrin toward *Saccharomyces cerevisiae*

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ABSTRACT

Sterilization activities of seven kinds of water-soluble dialkyloxo(tetraphenylporphyrinato)antimony(V) bromides (**1**) were examined against a yeast, *Saccharomyces cerevisiae*, under visible light irradiation. Sterilization activities were analyzed by the first-order kinetics involving the generation of singlet oxygen ($^1O_2$) in the complex between **1** and the cells. The formation of the complexes was confirmed by the measurements of the saturated adsorption concentrations of **1** inside yeast by absorption spectrophotometry using a confocal laser scanning microscope. The quantum yields of **1** for the generation of $^1O_2$ were determined to be 0.48–0.65. In order to ensure efficient sterilization, the requisite was that **1** were both water-soluble and oil-soluble. Presumably the oil-soluble feature of **1** was advantageous in passing through the cell wall of the yeast which consisted of hydrophobic peptideglucan and the water-soluble feature was advantageous in the adsorption occurring at hydrophilic sites inside the cell, causing fatal damage to the yeast.

1. Introduction

Bio-active chromophores have received much attention in connection with photodynamic therapy [1,2]. Porphyrins and metalloporphyrins are an attractive candidate as a bio active chromophore which will be selectively incorporated into specific microorganism sites [3–7]. Recently it has been elucidated that a cationic tetraphenylporphyrinatoantimony(V) complex can interact with single-stranded DNA to cause a guanine-selective damage to DNA through a singlet oxygen ($^1O_2$) mechanism under visible light irradiation [8]. Furthermore, we have reported that a cationic phosphorus(V) tetraphenylporphyrin complex can be adsorbed on *Escherichia coli* in an aqueous solution producing bactericidal activity under visible light irradiation [9]. Therefore, in order to perform more efficient biological reactions in aqueous solutions, we have developed the water-soluble (dialkyloxo)tetraphenylporphyrinatoantimony(V) bromides (**1**) (Scheme 1) whose solubility (C_W) in an aqueous solutions are listed in Table 1 [10]. In this paper we will investigate its sterilization activity against yeast cells of *Saccharomyces cerevisiae*.

2. Experimental

2.1. Materials

The preparations of **1a–c** were performed according to the reported method [3]. As a starting material for the preparation of alkyloxo(methoxo)tetraphenylporphyrinatoantimony(V) bromides (**1d–g**), bromo(methoxo)tetraphenylporphyrinatoantimony (V) bromide (**2**) was prepared from the partial methanolysis of dibromotetraphenylporphyrinatoantimony (V) bromide in MeOH–MeCN (1:1) at 80 °C [3]. The preparations of **1d–g** were performed by heating of a MeCN–pyridine solution (50:2, v/v, 52 ml) of **2** (0.2 g) with 1-alkanol (2 ml) at 120 °C until the Soret band shifted from 427 nm to 424 nm. After evaporation, the residue was dissolved into CH_2Cl_2. The CH_2Cl_2 solution was washed three times with aqueous HBr solution (50 ml) and purified by column chromatography on SiO_2 using $CHCl_3$–MeOH (9:1, v/v). ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were taken with a Bruker AV 400M spectrometer for $CDCl_3$ solutions using $SiMe_4$ as an internal standard. A matrix-assisted laser desorption/ionization mass spectra (MALDI-MS) were measured on a Bruker Daltonics

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Table 1
Properties of water-soluble porphyrins (**1**) in aqueous solution.

1	MW[a]	C_W (mM)[b]	C_O (mM)[c]	Soret band	Q-band		$[\mathbf{1}]_{ad}$ (mM)[g]
				ε (M^{-1} cm^{-1})[d]	λ_{max} (nm)[e]	ε (M^{-1} cm^{-1})[f]	
1a	848.4	0.08	0.038	5.62×10^5 [h]	549.8 (554.6)	2.14×10^4 [h]	6.7 ± 2.5
1b	862.4	0.10	0.041	5.01×10^5 [h]	550.5 (556.5)	1.95×10^4 [h]	11.4 ± 3.7
1c	876.5	0.13	0.025	5.01×10^5 [h]	550.2 (554.6)	1.86×10^4 [h]	33.2 ± 9.8
1d	946.6	1.09	0.149	3.20×10^5	549.8 (552.4)	1.16×10^4	54.2 ± 7.3
1e	1002.7	2.10	0.139	2.62×10^5	553.2 (553.3)	1.59×10^4	49.8 ± 6.2
1f	1030.4	2.21	0.156	2.88×10^5	552.8 (553.7)	1.32×10^4	22.5 ± 15.5
1g	1058.4	2.40	0.066	1.99×10^5	552.4 (nd [i])	1.24×10^4	nd[j]

[a] Molecular weight.
[b] Solubility (C_W) in water.
[c] Solubility (C_O) in dioxane.
[d] Molar absorptivity of **1** in the Soret band near 419 nm in an aqueous solution.
[e] Absorption maxima of **1** in the Q-band in an aqueous solution. The values in parenthesis were absorption maxima measured by a CLSM for **1** adsorbed on *S. cerevisiae*.
[f] Molar absorptivity of **1** in the Q-band in aqueous solution.
[g] Adsorption experiment was performed for an aqueous solution (2.5 ml) containing **1** (10 μM), *S. cerevisiae* (1×10^4 cell cm^{-1}), and agar (0.4 wt%). The saturated adsorption concentrations of **1** inside *S. cerevisiae* were determined by a CLSM absorption spectrophotometry.
[h] The values from a MeOH solution.
[i] Absorption maxima in *S. cerevisiae* was not determined due to its low concentration.
[j] Lower than the detection limit (1.65 mM).

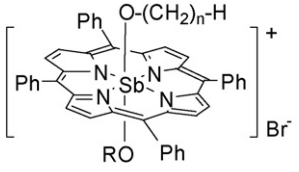
Autoflex III TOF/TOF in the positive ion mode at the Cooperative Research Center, University of Miyazaki.

2.1.1. Hexyloxo(methoxo)tetraphenylporphyrinatoantimony(V) bromide (*1d*)

Yield 31%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = −2.57 (t, J = 6.2 Hz, 2H), −2.19 (s, 3H), −2.01 to −1.94 (m, 2H), −1.63 to −1.56 (m, 2H), −0.35 (quint, J = 7.5 Hz, 2H), 0.32–0.39 (m, 5H), 7.92–8.02 (m, 12H), 8.27–8.30 (m, 4H), 8.34–8.37 (m, 4H), 9.56 (s, 8H); ^{13}C NMR δ = 13.46, 21.70, 22.81, 28.22, 29.79, 45.82, 58.00, 122.97, 127.96, 128.10, 130.03, 133.87, 134.71, 134.82, 138.14, 146.03; UV–vis (in MeOH) λ_{max}/nm (ε/10^4 M^{-1} cm^{-1}) 419 (39.8), 551 (1.62), 591 (0.93). Exact mass (MALDI-MS) calcd. for $C_{51}H_{44}N_4O_2Sb$ [M^+]: 865.2502. Found: 865.2429

2.1.2. Decyloxo(methoxo)tetraphenylporphyrinatoantimony(V) bromide (*1e*)

Yield 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = −2.57 (t, J = 6.1 Hz, 2H), −2.19 (s, 3H), −2.00 to −1.93 (m, 2H), −1.63 (quint, J = 7.6 Hz, 2H), −0.34 (quint, J = 7.6 Hz, 2H), 0.33 (quint, J = 7.6 Hz, 2H), 0.68 (quint, J = 7.6 Hz, 2H), 0.81 (t, J = 7.3 Hz, 3H), 0.89–0.96 (m, 2H), 1.00–1.07 (m, 2H), 1.16 (sextet, J = 7.3 Hz, 2H), 7.92–8.02 (m, 12H), 8.29 (d, J = 6.8 Hz, 4H), 8.36 (d, J = 6.8 Hz, 4H), 9.56 (s, 8H); ^{13}C NMR δ = 14.03, 22.54, 23.17, 27.67, 28.25, 28.82, 29.02, 29.03, 31.68, 45.86, 58.02, 122.96, 127.95, 128.11, 130.03, 133.87, 134.73, 134.83, 138.12, 146.01; UV–vis (in MeOH) λ_{max}/nm (ε/10^4 M^{-1} cm^{-1}) 419 (46.1), 551 (1.89), 590 (1.09). Exact mass (MALDI-MS) calcd. for $C_{55}H_{52}N_4O_2Sb$ [M^+]: 921.3128. Found: 921.3188



1a; R= H, n= 0 **1d**; R= Me, n= 6
1b; R= H, n= 1 **1e**; R= Me, n= 10
1c; R= Me, n= 1 **1f**; R= Me, n= 12
1g; R= Me, n= 14

Scheme 1. Water-soluble porphyrins (**1**).

2.1.3. Dodecyloxo(methoxo)tetraphenylporphyrinatoantimony(V) bromide (*1f*)

Yield 25%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = −2.57 (t, J = 6.2 Hz, 2H), −2.19 (s, 3H), −2.01 to −1.94 (m, 2H), −1.63 (quint, J = 7.6 Hz, 2H), −0.34 (quint, J = 7.6 Hz, 2H), 0.33 (quint, J = 7.6 Hz, 2H), 0.68 (quint, J = 7.6 Hz, 2H), 0.86 (t, J = 7.1 Hz, 3H), 0.92 (quint, J = 7.6 Hz, 2H), 1.01–1.08 (m, 2H), 1.10–1.30 (m, 6H), 7.92–8.02 (m, 12H), 8.28–8.30 (m, 4H), 8.35–8.37 (m, 4H), 9.56 (s, 8H); ^{13}C NMR δ = 14.03, 22.56, 23.11, 27.62, 28.18, 28.77, 29.04, 29.16, 29.32, 29.39, 31.76, 45.73, 57.94, 122.90, 127.90, 128.04, 129.97, 133.80, 134.67, 134.74, 138.05, 145.95; UV–vis (in MeOH) λ_{max}/nm (ε/10^4 M^{-1} cm^{-1}) 419 (37.4) 551 (1.54) 590 (0.90). Exact mass (MALDI-MS) calcd. for $C_{57}H_{56}N_4O_2Sb$ [M^+]: 949.3441. Found: 949.3458

2.1.4. Methoxo(tetradecyloxo)tetraphenylporphyrinatoantimony(V) bromide (*1g*)

Yield 25%. ^{1}H NMR (400 MHz, $CDCl_3$) δ = −2.57 (t, J = 6.2 Hz, 2H), −2.19 (s, 3H), −2.01 to −1.94 (m, 2H), −1.63 (quint, J = 7.6 Hz, 2H), −0.34 (quint, J = 7.6 Hz, 2H), 0.32 (quint, J = 7.6 Hz, 2H), 0.68 (quint, J = 7.6 Hz, 2H), 0.87 (t, J = 7.0 Hz, 3H), 0.91 (quint, J = 7.6 Hz, 2H), 1.01–1.08 (m, 2H), 1.09–1.32 (m, 10H), 7.91–8.02 (m, 12H), 8.28–8.29 (m, 4H), 8.34–8.37 (m, 4H), 9.56 (s, 8H); ^{13}C NMR δ = 14.08, 22.64, 23.18, 27.68, 28.26, 28.83, 29.10, 29.29, 29.38, 29.49, 29.56, 29.56, 31.86, 45.89, 58.02, 122.96, 127.94, 128.10, 130.02, 133.87, 134.72, 134.83, 138.12, 146.01; UV–vis (in MeOH) λ_{max}/nm (ε/10^4 M^{-1} cm^{-1}) 419 (40.8) 551 (1.59) 590 (0.91). Exact mass (MALDI-MS) calcd. for $C_{59}H_{60}N_4O_2Sb$ [M^+]: 977.3754. Found: 977.3729.

2.2. Spectral measurement

The molar absorption coefficiencies (ε) of **1** at the Soret and Q-bands were measured in aqueous solution with a Shimadzu V-550 spectrophotometer. The ε is summarized in Table 1. Microscopic spectrophotometry was performed with an Olympus FV-300 confocal laser scanning microscope (CLSM) equipped with a spectrophotometer (STFL 250, Seki Technotron) linked to a CLSM with an optical fiber.

The solubility of **1** in water was defined as the saturated concentration (C_W) by the following method [10]. The C_W of **1g** was measured with **1g** (5 mg) was suspended in pure water (1 ml) and left to stand for 3 days. The supernatant solution was moved to another vessel and diluted with MeOH to measure the absorption

Table 2
Visible light sterilization of *S. cerevisiae* with **1**.[a]

1	[**1**] (μM)[b]	Amount of bacteria ([*B*]) (cell ml^{-1})									k_2 (h^{-1})[d]
		t = 0 (min)[c]	10	20	30	40	50	60	80	100	120	
1a	0.05	[B_0] = 8500		8350		8050		7750	7600	7550	7000	0.09
1b	0.05	10400		10250		10000		9570	9600	9150	8400	0.09
1c	0.05	10800		10400		9730		9600	8700	7800	7200	0.18
1d	0.05	8000		2350		1070		550	130	150	250	2.71
1e	0.05	7870		4000		2200		1930	770	500	450	1.57
	0.04	10000		5450		4430		3170	1970	1400	1200	1.12
	0.03	10050		9550		9400		9700	9000	8970	9110	0.05
	0.02	10030		9800		9800		9630	9350	9600	9250	0.04
	0.01	8650		8350		8000		7600	7750	7230	7100	0.02
1f	0.05	9850	7230	3730	1570	430	150	200				3.79
1g	0.05	11700	8900	7300	2900	400	0	0				–[e]

[a] The sterilization was performed in an aqueous solution (10 ml) containing *S. cerevisiae* (1×10^4 cell ml^{-1}) with **1** by the irradiation of a fluorescent lamp (10.5 W cm^{-2}).
[b] Concentration of **1** used.
[c] Irradiation time in min.
[d] The rate constants (k_2) were obtained from the plots of ln([B_0]/[B]) vs. irradiation time.
[e] The rate constant (k_2) was determined to be 3.32×10^{11} cell mol^{-1} h^{-1} from the plots of [B_0] − [B] vs. irradiation time according to Eq. (5).

spectra of the solution. Solubility was defined as the saturated concentration (C_W) which was calculated using absorbance and ε_{419} (4.08×10^5 M^{-1} cm^{-1} in MeOH). The solubility (C_O) of **1a**–**g** in dioxane was determined in a similar manner to that used for C_W.

2.3. Determination of the quantum yields for the formation of $^1O_2$

The $^1O_2$ formation was directly measured by near-infrared luminescence around 1270 nm from deactivated $^1O_2$, which corresponds to the $^1O_2$ ($^1\Delta_g$) – $^3O_2$($^3\Sigma_g^-$) transition. A direct detection system which consisted of an Nd:YAG laser (THG/355 nm, 30 Hz; Tempest-30, New Wave Research) as an excitation light source (355 nm, intensity: 280 mW cm^{-2}), a quartz cuvette as an irradiation cell, a spectroscope (250is, Chromex, Albuquerque, NM, USA), and a near-infrared gated multi-channel detector ICCD camera (NIR-II, Hamamatsu Photonics, Shizuoka, Japan) was built in Photon Medical Research Center, Hamamatsu University School of Medicine [11,12]. The gate time and accumulation time were 5–50 μs after the laser pulse and 128 s (total: 36 J cm^{-2}), respectively. The sample solutions containing 8 μM of **1a**, **1c**, or **1e** in a 3.5 ml D_2O solution were measured with the above system. The quantum yields of $^1O_2$ formation were estimated from the comparison of the $^1O_2$ emission intensities by **1** and methylene blue (singlet oxygen quantum yield: 0.52 in D_2O) [13].

2.4. Preparation of cell suspension

S. cerevisiae NBRC 2044 was cultured aerobically at 30 °C for 8 h in a basal medium (pH 6.5) consisting of bactotryptone (10 g dm^{-3}, Difco), yeast extract (5 g dm^{-3}), and NaCl (10 g dm^{-3}). After centrifugation of the cultured broth for 10 min, the harvested cells were washed and suspended in water to create a cell suspension. Cell numbers were measured with a hemocytometer and the amounts of *S. cerevisiae* in the suspension were adjusted to ca. 2.5×10^5 cell ml^{-1}.

3. Results and discussion

3.1. Photochemical sterilization

Many microorganisms usually cannot survive in pure water because the osmotic pressure of the microorganism is higher. Therefore, a usual treatment of microorganism was performed in buffer solution. However, the solubility of **1** was very low in the presence of both NaCl and phosphate buffers. As a microorganism, therefore, we selected a yeast, *S. cerevisiae*, which can survive in pure water. The sterilization activities of **1** were examined in an L-type glass tube in a manner similar to the reported method [12]. A cell suspension of *S. cerevisiae* (1 ml, ca. 10^5 cell ml^{-1}), an aqueous solution of **1** (0.1 ml, 5.0 μM), and water (8.9 ml) were introduced into L-type glass tubes. The prepared aqueous solution (10 ml) containing yeast cells (1×10^4 cell ml^{-1}) and **1** (0.05 μM) was subjected to the sterilization experiments. The photochemical sterilization experiment was performed in an L-type tube by irradiation with a fluorescent lamp (Panasonic FL-15ECW, λ = 400–723 nm, the maximum intensity: 545 nm, 10.5 W cm^{-2}) on a reciprocal shaker in a manner similar to the reported method [9]. A portion (0.1 ml) of the reaction mixture was taken at 10 or 20 min intervals and plated on an agar medium. The amount of living cells ([B]) was defined as the average number of colonies of *S. cerevisiae* which appeared after incubation for 36 h at 30 °C in three replicate plates. The results are summarized in Table 2. As a typical example of a time course is shown in Fig. 1, [B] decreased with an increase in irradiation time in the presence of **1e**. In contrast, the control experiments in the presence of **1e** under dark conditions maintained [B] at nearly original levels.

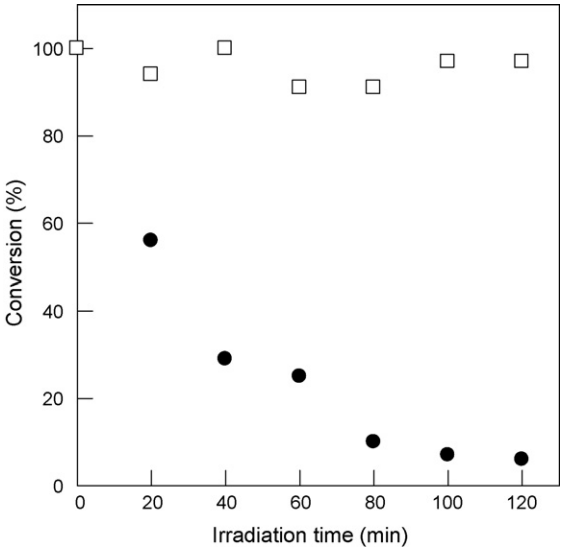


Fig. 1. Time-conversion plots of conversion (100[B]/[B_0]) for the sterilization with **1e** under irradiation (●) and dark (□) conditions. [**1e**] = 0.05 μM.

3.2. CLSM-analysis of the concentration of **1** adsorbed on S. cerevisiae

The interaction of **1** with yeast cells was examined by quantitative analysis of **1** incorporated into the yeast cells with a CLSM [14]. An aqueous solution of **1** (0.5 ml, 50 μM) was added to the cell suspension (1.0 ml; ca. 2.5×10^4 cell ml^{-1}) of *S. cerevisiae*, and then an aqueous solution of agar (1 wt%; 1.0 ml) was added into the solution in order to stop the Brownian motion of cells during the CLSM analysis. A portion of the prepared aqueous solution containing **1** (10 μM, $[\mathbf{1}]_{out}$), *S. cerevisiae* (ca. 1.0×10^4 cell ml^{-1}), and agar (0.4 wt%) was taken on a space (1 cm × 1 cm) surrounded by silicone spacer (thickness 50 μm) put on a slide glass. The slide glass was set on the stage to be subjected to absorption spectrophotometry with a CLSM. In order to measure the saturated adsorption concentration ($[\mathbf{1}]_{ad}$), $[\mathbf{1}]_{out}$ were set to 10 μM which were much higher than the concentration in the sterilization experiments (usually 0.05 μM). It was confirmed that $[\mathbf{1}]_{ad}$ were the saturated concentration since $[\mathbf{1}]_{ad}$ reached to the maximum concentration when $[\mathbf{1}]_{out} \geq 10$ μM [14].

The saturated adsorption concentrations of **1** ($[\mathbf{1}]_{ad}$) on *S. cerevisiae* were determined by absorption spectrophotometry at the Q-band using molar coefficiency (ε; Table 1), path length (b), and absorbance (A) according to Lambert–Beer's law: $A = \varepsilon\, b[\mathbf{1}]_{ad}$. b was determined to be 4.33 ± 0.51 μm from the CLSM fluorescence image where the fluorescence was emitted from the inside rather than the walls of cells. $[\mathbf{1}]_{ad}$ are listed in Table 1. In the cases of **1a**–**f**, many amounts of **1** were adsorbed to *S. cerevisiae*. In the case of **1g**, $[\mathbf{1g}]_{ad}$ was less than a measurable lower limit (1.65 mM).

3.3. Analysis of sterilization reaction

It has been reported that the inactivation effects of **1a** have been attributed to a damage caused by $^1O_2$ generated by photosensitization of **1a** [3]. The $^1O_2$ was formed by an intersystem crossing from the singlet to the triplet states of **1a** and the subsequent energy transfer from $^3\mathbf{1a}^*$ to $^3O_2$. Therefore, it can be assumed that the generation of $^1O_2{}^*$ was responsible for the sterilization by **1b**–**g**. The quantum yields for the formation of $^1O_2$ were determined to be 0.48 (**1a**), 0.65 (**1c**), and 0.53 (**1e**), showing no large difference among **1**.

As a working hypothesis, we postulated a Michaelis–Menten type of mechanism (Scheme 2) where the sterilization occurred at the complex (**1**•*B*) between the **1** and cells (*B*). In the cases of **1a**–**f**, it was safely concluded from the results of the absorption spectrometry described above that all cells included considerable amounts of **1**. This showed that the amount of *B* ([*B*]) was equal to that of the **1**•*B* ([**1**•*B*]); i.e. [*B*] = [**1**•*B*]. Therefore, the sterilization was obeyed the first-order kinetics (Eq. (1)). According to Eq. (1), $\ln([B_0]/[B])$ are proportional to the irradiation time (t) where $[B_0]$ represents the initial concentration of *S. cerevisiae* (Eq. (2)). Indeed, the plots of $\ln([B_0]/[B])$ vs. t gave straight lines with the slope of k_2 (Fig. 2). The k_2 are listed in Table 2. The k_2 were depended on the [**1**] used, as shown in the case of **1e**. Therefore, the sterilization activities of **1a**–**f** were compared by k_2 at [**1**] = 0.05 μM.

In the case of **1g**, however, the kinetics did not follow the Eq. (2). In this case, all of the cell could not include a considerable amount of **1g** because the amount of $[\mathbf{1g}]_{ad}$ was low. The bactericidal reaction rate (v) was represented by the Eq. (3) where K_m represents $(k_{-1} + k_2)/k_1$. If k_1 is much larger than k_{-1} and k_2, then v can be represented by Eq. (4). k_2 can be derived from the slope (k_2[C]) of the plot of $[B_0] - [B]$ vs. t according to Eq. (5). When [**1g**] = 0.05 μM, k_2 was determined to be 3.32×10^{11} cell mol^{-1} h^{-1} from the plots of $B_0 - B$ vs. t (Fig. 3).

$$v = \frac{-d[B]}{dt} = k_2[B] \quad (1)$$

$$\ln\left(\frac{[B_0]}{[B]}\right) = k_2 t \quad (2)$$

$$v = \frac{k_2[B][\mathbf{1}]}{(K_m + [B])} \quad (3)$$

$$v = \frac{-d[B]}{dt} = k_2[\mathbf{1}] \quad (4)$$

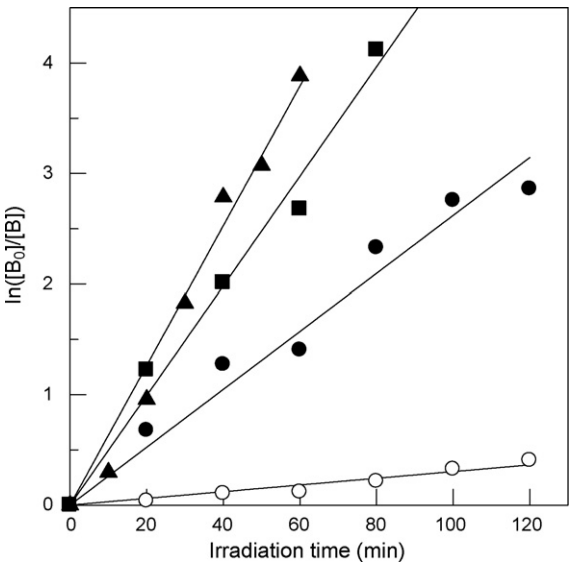


Fig. 2. Plots of $\ln([B_0]/[B])$ vs. irradiation time in the sterilization of *S. cerevisiae* with **1c** (○), **1d** (■), **1e** (●), and **1f** (▲)under visible light irradiation. Conditions: [**1**] = 0.05 μM.

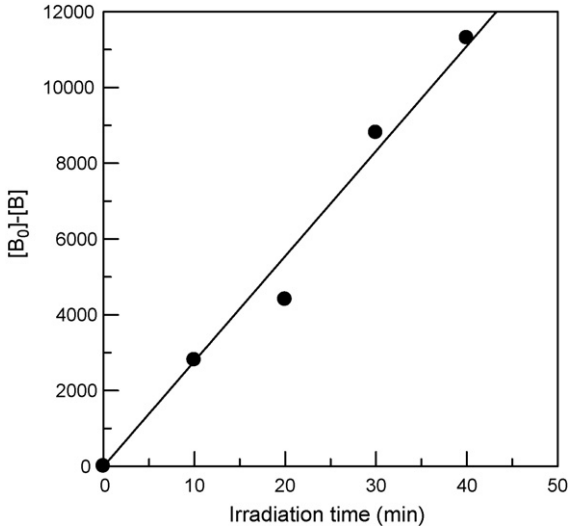
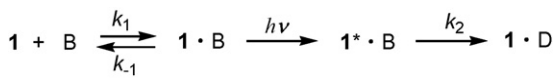


Fig. 3. Plots of $[B_0]$–$[B]$ vs. irradiation time in the sterilization of *S. cerevisiae* with **1g** (0.05 μM) under visible light irradiation.



Scheme 2. Sterilization mechanism of **1**. B: living cells of *S. cerevisiae*, D: inactivated cells.

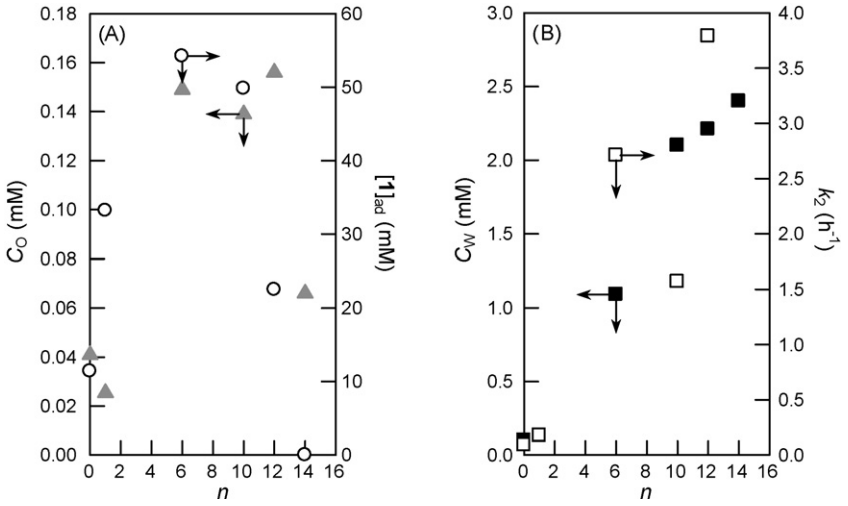


Fig. 4. Dependence of (A) C_O (▲) and $[\mathbf{1}]_{ad}$ (○) on the number of the methylene units (n) on the alkyloxo ligand of **1b**–**g** and (B) dependence of C_W (■) and k_2 (□) on n.

$$[B_0] - [B] = k_2[\mathbf{1}]t \quad (5)$$

4. Conclusions

As shown in Fig. 4A, **1** with a higher C_O had higher $[\mathbf{1}]_{ad}$. This suggested that oil-soluble **1** could easily pass through cell wall which consisted of hydrophobic peptideglucan. Therefore, the sterilization was inefficient in the cases of **1a** and **1b** whose $[\mathbf{1}]_{ad}$ were low. Also in the case of **1c** whose $[\mathbf{1c}]_{ad}$ were high, however, the sterilization was inefficient. In this case, the **1c** was less soluble in water (C_W = 0.13 mM). Therefore, water-soluble **1** were required for efficient sterilization as shown in Fig. 4B. Indeed, the sterilization reaction occurred efficiently in the cases of **1d**–**g** which were water-soluble (C_W > 1.00 mM). It was suggested that the attack of $^1O_2$ on the hydrophilic parts such as mitochondria and nuclear caused the fatal damage of the cells rather than that of hydrophobic parts. As a consequence, the $^1O_2$-sensitizers having both properties of water-soluble and oil-soluble were required for efficient sterilization.

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