Chapter

Electron Transfer-Supported Photodynamic Therapy

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Abstract

Photodynamic therapy (PDT) is a less-invasive treatment of cancer and precancerous lesions. Porphyrin derivatives have been used and studied as the photosensitizers for PDT. In general, the biomacromolecules oxidation by singlet oxygen, which is produced through energy transfer from the photoexcited photosensitizers to oxygen molecules, is an important mechanism of PDT. However, the traditional PDT effect may be restricted, because tumors are in a hypoxic condition and in certain cases, PDT enhances hypoxia via vascular damage. To solve this problem, the electron transfer-mediated oxidation of biomolecules has been proposed as the PDT mechanism. Specifically, porphyrin phosphorus(V) complexes demonstrate relatively strong photooxidative activity in protein damage through electron transfer. Furthermore, other photosensitizers, *e.g.*, cationic free-base porphyrins, can oxidize biomolecules through electron transfer. The electron transfer-supported PDT may play the important roles in hypoxia cancer therapy. Furthermore, the electron transfer-supported mechanism may contribute to antimicrobial PDT. In this chapter, recent topics about the biomolecules photooxidation by electron transfer-supported mechanism are reviewed.

Keywords: Photoinduced electron transfer, porphyrin phosphorus(V) complex, protein oxidation, cationic porphyrin, phenothiazine dyes

1. Introduction

Photodynamic therapy (PDT) is a less-invasive treatment of cancer and other nonmalignant conditions [1–3]. This treatment is a medicinal application of photochemistry. Antimicrobial treatment, called as antimicrobial photodynamic therapy (aPDT) or photodynamic antimicrobial chemotherapy (PACT), is also important application [4–7]. In the case of cancer treatment, less-toxic PDT reagents, photosensitizers, cause oxidative damage to biomolecules, including protein, nucleic acids, and/or other compounds, under visible-light irradiation. This photosensitized reaction results in necrosis or apoptosis of cancer cells [1–3]. As the PDT photosensitizers, porphyrins have been extensively studied and used [8–11]. For example, porfimer sodium [12, 13] and talaporfin sodium [13], an oligomer and a monomer of a free-base anionic porphyrin, respectively, are well-known photosensitizers in clinical use. In general, the porphyrin photosensitizer (*e.g.*, almost 60 mg/body for talaporfin sodium) is given for the target tissue, followed by irradiation of the visible light (e.g., 664 nm, 150 mW cm^{-2}, and 10 J cm^{-2}). To reduce the risk of adverse side effects, the development of efficient photosensitizers that work with harmless weak light is important. Furthermore, consideration of PDT mechanism is also important to develop effective photosensitizer. Most of porphyrins have relatively large quantum

yield (Φ_Δ) for singlet oxygen ($^1O_2$), a reactive oxygen species (ROS), generation [14]. $^1O_2$ can be easily generated by relatively small energy photon of long wavelength visible light and/or near infrared radiation (wavelength $\gtrsim$ 770 nm) through energy transfer from photoexcited photosensitizer to oxygen molecule [15–17]. Radiation in the long wavelength region called "optical window", 600 ~ 1300 nm, can penetrate human tissue deeply [18]. Therefore, $^1O_2$ is the important reactive species of porphyrin-based PDT. However, the phototoxic effect of $^1O_2$ on PDT is restricted because of the hypoxic condition of tumors [19–22]. Furthermore, in certain cases, PDT itself enhances hypoxia [23] via vascular damage [24]. This "hypoxia problem" of tumor is very important to improve the PDT effect.

Oxidation is defined as the oxygenation, hydrogen extraction, and electron extraction. Electron extraction from biomolecules to photoexcited photosensitizer is also the mechanism of oxidative biomolecule damage. This electron transfer oxidation may be an important mechanism to resolve the "hypoxia problem" and to develop the effective PDT photosensitizers. Phosphorus(V) porphyrins [25, 26] and cationic free-base porphyrins [27] have relatively strong oxidative activity through electron transfer [28]. Furthermore, electron transfer process can be control by surroundings condition, for example pH of medium [29, 30].

In this chapter, recent studies about the electron transfer-supported photosensitizer for PDT are reviewed. The examples of activity control of photosensitizer for the cancer-selective PDT are also introduced. In the last section, the role of electron transfer mechanism in aPDT is discussed.

2. Electron transfer oxidation as a mechanism of photosensitized biomolecule damage

In general, photosensitized biomolecule damage can be explained by oxygen-independent mechanism (Type I mechanism) and oxygen-mediated mechanism (Type II mechanism) (**Figure 1**) [31–33]. Because the electron transfer-mediated biomolecule oxidation does not absolutely require oxygen, this mechanism is categorized as Type I mechanism. On the other hand, biomolecule oxidation through $^1O_2$ generation is defined as Type II mechanism (Type II, major). Another ROS-mediated process, superoxide ($O_2^{\bullet-}$)-mediated biomolecule oxidation is also categorized as the Type II mechanism (Type II, minor). Although $O_2^{\bullet-}$ is produced through electron transfer from photoexcited photosensitizer, it's not categorized as the Type I mechanism. The initial process of electron transfer-mediated biomolecule oxidation is an electron extraction from the targeting biomolecule, such as protein, to the photoexcited photosensitizer.

2.1 Driving force dependence of electron transfer

The driving force of electron transfer, Gibbs energy (ΔG), is determined by the excitation energy of photosensitizer (photon energy) and the redox potential of photosensitizer and targeting biomolecule. The electron transfer is a relaxation process of photoexcited photosensitizer. Fast electron transfer is advantageous for an efficient electron transfer. Due to the Marcus theory [34, 35], the rate constant of electron transfer (k_{ET}) is expressed using ΔG as follows:

$$k_{ET} = \sqrt{\frac{4\pi^3}{h^2 \lambda K_B T}} V_{DA}{}^2 \exp \frac{-\left(\Delta G^* + \lambda\right)^2}{4\lambda K_B T}, \quad (1)$$

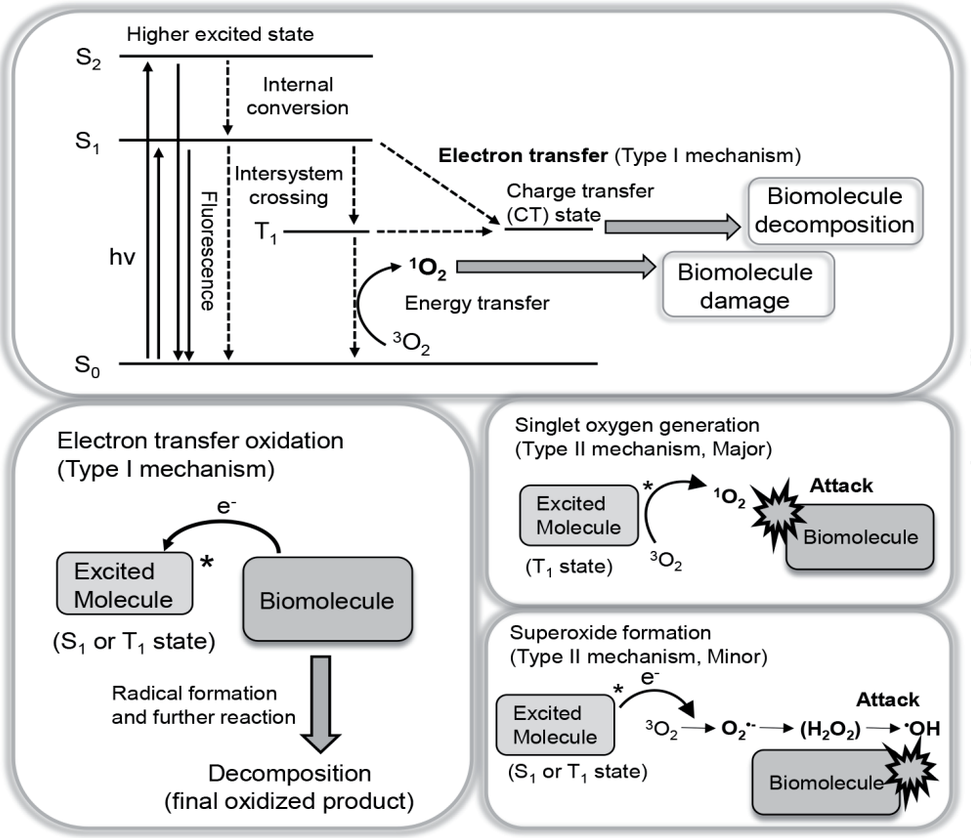


Figure 1.
Relaxation process of photoexcited state of photosensitizer and the typical photosensitized biomolecule damaging mechanisms.

where h is Plank constant, λ is the reorganization energy, K_B is the Boltzmann constant, and V_{DA} is the effective electronic Hamiltonian matrix element. The λ can be calculated from the following equation:

$$\lambda = \frac{e^2}{4\pi\varepsilon_0}\left(\frac{1}{2r_D}+\frac{1}{2r_A}+\frac{2}{d}\right)\left(\frac{1}{n^2}-\frac{1}{\varepsilon}\right), \tag{2}$$

where e is the elementary charge, ε_0 is the vacuum permeability (8.854×10^{-12} F m^{-1}), r_D and r_A are the radius of the electron donor and that of acceptor, respectively, d is the distance between electron donor and acceptor, n is the refractive index, and ε is the static dielectric constant of surrounding material. Since the V_{DA} is determined by the overlap between wavefunctions of electron donor and acceptor, the electron transfer rate strongly depends on the d, and decreased exponentially with an increase in d. Therefore, association between photosensitizer and targeting biomolecule is very important. The ΔG, driving force of electron transfer, is expressed as follows:

$$\Delta G = e\left(E_{red} - E_{ox}\right) - E_{0-0}, \tag{3}$$

where E_{red} is the redox potential of a one-electron reduction of photosensitizer, E_{ox} is the redox potential of a one-electron oxidation of targeting biomolecule, and E_{0-0} is the 0–0 energy (singlet excited (S_1) energy) of photosensitizer. The Eq. (1) indicates that k_{ET} becomes maximum at $\Delta G = \lambda$. However, in general, large -ΔG is

advantageous for fast electron transfer. Therefore, small (small absolute value) E_{red} and/or large (large absolute value) E_{ox} is appropriate for effective electron transfer. To evaluate the electron transfer in the triplet excited (T_1) state, the "E_{0-0}" term in Eq. (3) is replaced with the T_1 state energy. Because T_1 state energy is smaller than E_{0-0}, in general, electron transfer oxidation by T_1 state photosensitizer becomes difficult.

2.2 Excitation energy and electron transfer

Excitation energy (photon energy) strongly affects the electron transfer rate and efficiency as the Eq. (3). Indeed, an ultraviolet photosensitizer can oxidize DNA, which is relatively resistant to the electron extraction, through photoinduced electron transfer [32, 33]. However, ultraviolet radiation is harmful for human tissue. Furthermore, long wavelength visible light or near infrared radiation can penetrate human tissue deeply as mentioned above as the optical window [18]. Therefore, visible light (or near infrared) photosensitizer, such as porphyrins and phthalocyanines, are important for PDT. To realize the electron transfer photosensitizer, which can be excited by long wavelength light, the design and synthesis of photosensitizer molecules with small E_{red} value are required. However, a molecule with small E_{red} has tend to decay through reduction by surrounding molecules, and small E_{red} is not appropriate for stability of molecule.

2.3 Kinetics of electron transfer

In general, electron transfer can be demonstrated by a transient absorption spectrum measurement [36, 37] and a time-resolved electron paramagnetic resonance measurement [38, 39]. The k_{ET} values can be determined by the analysis of transient absorption spectra. Fluorescence lifetime measurement is also an important method [40]. Although fluorescence lifetime is affected by various factors other than electron transfer, it is sensitive and convenient method. If other factors can be excluded, this method is advantageous for the kinetic evaluation of electron transfer. The k_{ET} value can be obtained using fluorescence lifetime by the following equation:

$$k_{ET} = \frac{1}{\tau_f} - \frac{1}{\tau_f^0}, \tag{4}$$

where τ_f is the observed fluorescence lifetime of photosensitizer with electron donor (targeting biomolecule) and τ_f^0 is that without electron donor. In general, k_{ET} becomes larger than $10^8 \sim 10^9$ s^{-1} in the case of electron transfer in the S_1 state, because lifetime of most of porphyrin S_1 state is order of several nanosecond. In the case of T_1 state, the lifetime is order of microsecond and the rate constant becomes relatively small. As mentioned above, the T_1 state is not appropriate for electron transfer oxidation from the thermodynamic point of view.

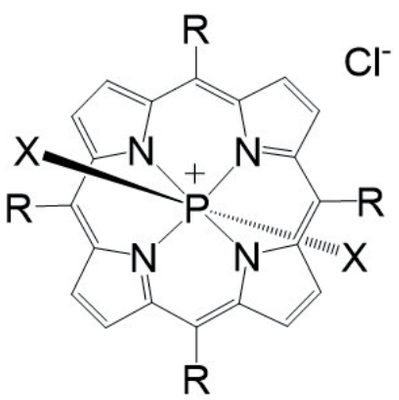
3. Phosphorus(V) porphyrin photosensitizer

Porphyrin derivatives have been used as clinical photosensitizer for PDT [8–11]. Porfimer sodium [12, 13] and Talaporfin sodium [13] are famous examples of clinically used photosensitizers. The PDT mechanism of these porphyrins is $^1O_2$ generation. The photochemical property of porphyrin can be changed by the replacement of the central atom and substitution. It has been reported that phosphorus(V)

porphyrin can oxidize biomolecules, such as nucleobase [41], protein [42–48], and other biomolecules [49, 50] through electron transfer.

3.1 General property of phosphorus(V) porphyrin

General procedure of synthesis method of phosphorus(V) porphyrin is a reflux of free base porphyrin with phosphoryl chloride in dry pyridine [51]. The photochemical property of phosphorus(V) porphyrin can be improved by the substitution of the *meso*- or *β*-positions and the axial ligand (**Figure 2**) [42–53]. An example of phosphorus(V) porphyrin, diethoxyP(V)tetrakis(4-methoxyphenyl) porphyrin chloride, is shown in **Figure 3**. The calculation with density functional theory (DFT) at ωB97X-D/6-31G* level shows the distorted structure of phosphorus(V) porphyrin. Their distorted structures have been reported from the results of X-ray crystal analysis [54]. Phosphorus(V) porphyrins introduced in this chapter are listed in **Table 1**. Because phosphorus(V) porphyrin is a cationic



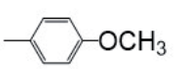
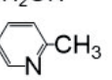
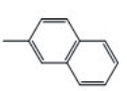
Compounds	R	X
Por1		-Cl
Por2		$-OCH_3$
Por3		$-OCH_2CH_3$
Por4		$-OCH_2CF_3$
Por5		$-OCH_2CH_2OH$
Por6		$-OCH_2CH_2CH_2OH$
Por7		
Por8		-Cl
Por9		$-OCH_3$
Por10		$-OCH_2CH_3$
Por11		$-OCH_2CF_3$
Por12		$-OCH_2CH_2OH$
Por13		
Por14		$-OCH_3$

Figure 2.
Structures of phosphorus(V) porphyrins.

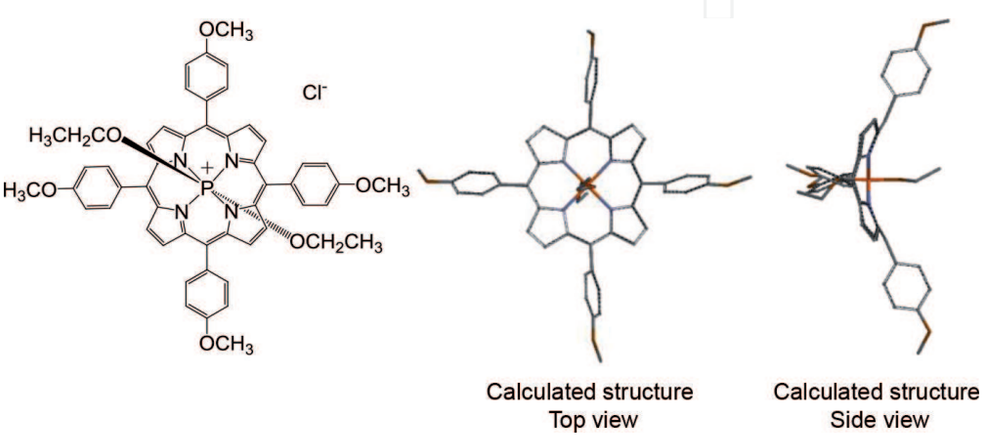


Figure 3.
Optimized structure of **Por10** *by the DFT calculation at ωB97X-D/6-31G* level.*

porphyrin, its water solubility is relatively large. Furthermore, hydrophilic substitution markedly increases the water solubility [55]. One of the most important characteristics of phosphorus(V) porphyrin is relatively small E_{red} value due to the positive charge of the central phosphorus atom, resulting in the strong oxidative activity in the photoexcited state. This character is very important as electron transfer-supported photosensitizer for PDT. Furthermore, in general, phosphorus(V) porphyrin has relatively large quantum yield of photosensitized $^1O_2$ generation in an aqueous solution (Φ_Δ is more than 0.5, **Table 1**) due to the effective intersystem crossing [42–47]. In the presence of enough oxygen molecules, phosphorus(V) porphyrin can oxidize biomolecule through $^1O_2$ generation, a traditional PDT mechanism.

3.2 Photosensitized protein damage by phosphorus(V) porphyrin through electron transfer

Isolated amino acids, a water-soluble protein, and enzymes have been used as the targeting biomacromolecules to examine photosensitizer activity of phosphorus(V) porphyrins [42]. For example, human serum albumin (HSA), a water-soluble protein, is a convenient target. The crystal structure and amino acid sequence of HSA have been clarified [56]. In addition, HSA has major drug specific binding sites identified as Sudlow's site I and site II [57]. The mono-cationic phosphorus(V) porphyrins listed in **Table 1** are well-soluble in organic solvents (*e.g.*, alcohol) rather than water, indicating the hydrophobic character beside the hydrophilicity. Therefore, binding interaction between HSA and phosphorus(V)

Compounds	E_{red} / V	E_{0-0} / eV	Φ_f	Φ_Δ	Ref.
Por1	−0.30	2.04, $PBS_{1.25EtOH}$		0.96, EtOH	[42]
Por2	−0.50[a]	2.03, $PBS_{1.25EtOH}$[a] 2.03, PBS[b]	0.017, $PBS_{2.5EtOH}$[c] 0.023, EtOH[c]	0.64, PBS[b] 0.93, $PBS_{2.5EtOH}$[c]	[42][a], [43][b], [52][c]
Por3	−0.54[e]	2.04, PBS[e]		0.59, PBS[d]	[44][d], [50][e]
Por4	−0.40[e]	2.03, PBS[e]		0.68, PBS[d]	[44][d], [50][e]
Por5	−0.51	2.03, PBS	0.048, PBS	0.88, PBS	[45]
Por6	−0.51	2.03, PBS	0.043, PBS	0.80, PBS	[45]
Por7	−0.54	2.02, $PBS_{1.25EtOH}$		0.94, EtOH	[42]
Por8	−0.33		0.029, PBS	0.97, PBS	[46]
Por9	−0.58		0.024, PBS	0.86, PBS	[46]
Por10	−0.58	1.96, $PBS_{1.0EtOH}$	0.067, EtOH	0.84, EtOH	[47]
Por11	−0.43	1.98, $PBS_{1.0EtOH}$	0.086, EtOH	0.82, EtOH	[47]
Por12	−0.57		0.029, PBS	0.83, PBS	[46]
Por13	−0.55	2.01, $PBS_{1.0EtOH}$	pH-dependent	pH-dependent	[48]
Por14		2.00, $PBS_{2.5EtOH}$	0.034, EtOH	ND, $PBS_{2.5EtOH}$	[52]

E_{red}: measured in acetonitrile (vs. saturated calomel electrode; SCE), PBS: 10 mM sodium phosphate buffer (pH 7.6) solution, EtOH: ethanol, $PBS_{ETOH2.5}$: PBS containing 2.5% ethanol, $PBS_{ETOH1.25}$: PBS containing 1.25% ethanol, $PBS_{ETOH1.0}$: PBS containing 1.0% ethanol, Φ_f: Fluorescence quantum yield. ND: not detected.

Table 1.
Examples of phosphorus(V) porphyrin photosensitizers and their photochemical properties.

porphyrins is expected and their binding site can be speculated. Because the electron transfer-mediated oxidation strongly depends on the distance between photosensitizer and the target molecule, a binding interaction is very important. HSA has one tryptophan, which is easily oxidized by oxidative stress, including $^1O_2$ and electron transfer reaction [42–47, 58]. Tryptophan can emit relatively strong fluorescence and its damage can be detected by fluorescence measurement [45, 58]. Using these characteristics of HSA, the oxidative damage of tryptophan residue by photosensitized reaction can be easily examined by a fluorometry [45–47, 58].

Qualitative study of HSA photodamage by phosphorus(V) porphyrins was reported using **Por2** [43]. **Por2** oxidized the tryptophan of HSA through $^1O_2$ generation and electron transfer. It has been considered that damaged tryptophan is changed to *N*-formylkynurenine and other decomposed products [59, 60]. $^1O_2$ can oxidize the tryptophan residue of HSA [61]. Using isolated amino acids, it has been demonstrated that tyrosine and tryptophan can be oxidized by photoexcited **Por2** [42].

Photosensitized HSA damage by **Por5** and **Por6** was quantitatively clarified [45]. **Por5** and **Por6** bound to HSA and damaged its tryptophan residue during photoirradiation. **Por5** and **Por6** photosensitized $^1O_2$ generation, and the contribution of $^1O_2$ was confirmed by the inhibitory effect of a $^1O_2$ quencher, sodium azide (NaN_3, [62]). From the kinetic analysis, the contribution of electron transfer mechanism to HSA damage was demonstrated [45]. Fluorescence lifetime measurement and the calculation of ΔG supported the electron transfer mechanism.

To realize the effective PDT photosensitizer, response of photosensitizers to long wavelength visible light or near infrared region is important. To improve the abovementioned phosphorus(V) porphyrins, **Por5** and **Por6**, *meso*-phenyl substituted derivatives were designed and synthesized [46]. **Por8**, **Por9**, and **Por12** can be excited under the irradiation of long-wavelength visible light (> 630 nm). These phosphorus(V) porphyrins induced tryptophan oxidation in HSA under illumination with light-emitting diode (central wavelength: 659 nm), and this protein photodamage was barely inhibited by NaN_3 [46]. Fluorescence lifetimes of phosphorus(V) porphyrins was decreased by HSA, suggesting the electron transfer quenching. The ΔG value of electron transfer from tryptophan to the S_1 state of these porphyrins calculated from their redox potentials also supported the electron transfer-mediated oxidation.

3.3 Cancer selective photodynamic action of phosphorus(V) porphyrin photosensitizers

Above mentioned phosphorus(V) porphyrins, **Por8**, **Por9**, and **Por12**, exhibited the cancer cell selective toxicity under visible light irradiation [46].

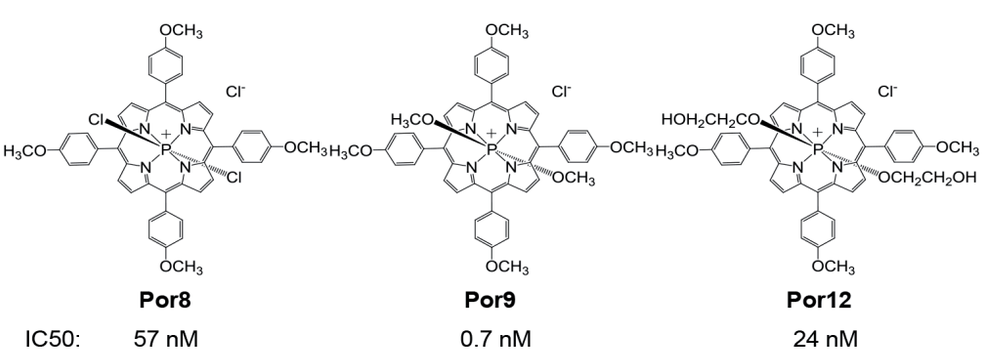


Figure 4.
Structures of ***Por8, Por9****, and* ***Por12****, and their IC50 values for HeLa cells under photoirradiation [46].*

Photocytotoxicity to HeLa cells by these porphyrins are the following order: **Por9** > **Por12** > **Por8** in the condition of previous report (**Figure 4**) [46]. Although the half maximal inhibitory concentration (IC50) value for **Por8** is largest (least phototoxicity) in the three phosphorus(V) porphyrins, its photocytotoxicity to cancer cells is sufficiently high. Furthermore, **Por8** did not exhibit photocytotoxicity to HaCaT cells, cultured human skin cells (normal cell model). **Por9** and **Por12** exhibited phototoxicity to HaCaT cells, however, these IC50 value were significantly larger than those for HeLa cells and cellular DNA damage in HaCat cells were not observed. These three phosphorus(V) porphyrins demonstrated significant PDT effects on mice tumor models [46]. The observed PDT effects by these porphyrins are almost the same, and are comparable with that of talaporfin sodium. These results suggest the cancer selectivity of **Por8**, **Por9**, and **Por12**, and lower carcinogenic risk to normal cells. Specifically, **Por8**, of which the redox potential is most advantageous for the electron transfer-mediated biomolecule oxidation, demonstrated the highest cancer-selectivity and significant PDT effect under irradiation with long-wavelength visible light.

3.4 Photoinduced electron transfer by phosphorus(V) porphyrin triggers the chain reaction for NADH decomposition

The electron transfer mechanism can contribute to oxidation other various biomolecules. For example, nicotinamide adenine dinucleotide (NADH), an important endogenous reductant, becomes an important targeting molecule [50]. The S_1 states of **Por3** and **Por4** easily extract electron from NADH, resulting in the formation of

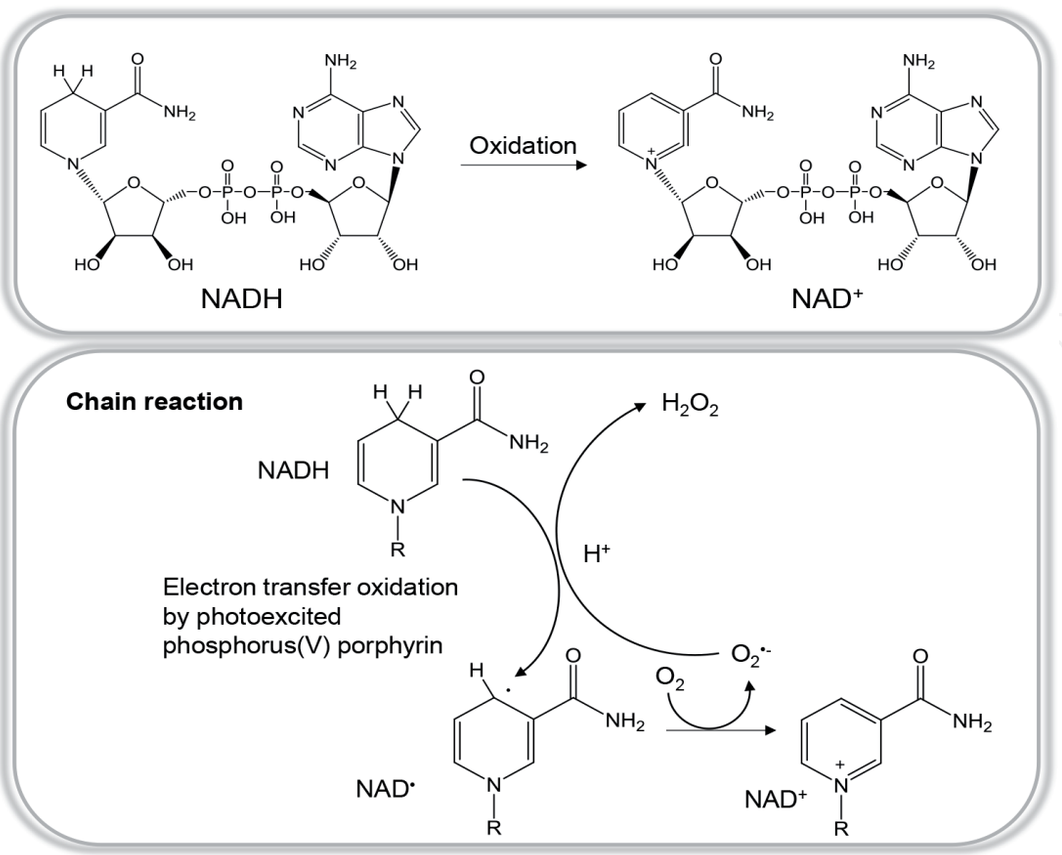


Figure 5.
Structures of NADH and its oxidized form, and the electron transfer-triggered chain reaction of NADH decomposition.

$NAD^{\bullet}$, a radical. Further oxidation leads to the irreversible decomposition of NADH to NAD^{+} (**Figure 5**). The total quantum yield of NADH decomposition (Φ_D) is expressed as follows:

$$\Phi_D = \Phi_{ET} \times \Phi_{FR}, \tag{5}$$

where Φ_{ET} is the quantum yield of the initial process (electron transfer) and Φ_{FR} is that of the further reaction to form NAD^{+}. Analysis of the quantum yields, obtained values of Φ_{FR} became much larger than unity. These findings suggest that the electron accepting by the photoexcited **Por3** and **Por4** triggers a chain reaction of NADH oxidation (**Figure 5**). The initial electron transfer to photoexcited **Por3** or **Por4** produces $NAD^{\bullet}$. The $NAD^{\bullet}$ immediately reacts with molecular oxygen to produce $O_2^{\bullet-}$:

$$NAD^{\bullet} + O_2 \rightarrow NAD^{+} + O_2^{\bullet-}. \tag{6}$$

In the following process, $O_2^{\bullet-}$ oxidizes NADH and hydrogen peroxide (H_2O_2) is produced [63]:

$$NADH + O_2^{\bullet-} + H^{+} \rightarrow NAD^{\bullet} + H_2O_2. \tag{7}$$

The electron transfer-mediated reaction induces the chain reaction, resulting in the acceleration of NADH decomposition and secondary generation of reactive oxygen species. In the case of direct photosensitized reaction, ultraviolet photon is required to produce H_2O_2 [28]. The secondary formed H_2O_2 may produce hydroxyl radicals ($^{\bullet}OH$), very strong ROS. These results suggest that electron transfer reaction with visible light irradiation induces a severe toxic effect through a chain reaction and the formation of H_2O_2, similarly to the ultraviolet radiation.

3.5 Photosensitized oxidation of folic acid by phosphorus(V) porphyrin through electron transfer

Folic acid, a vitamin, is also oxidized through photoinduced electron transfer [64]. Because the fluorescence intensity of folic acid is significantly increased by the decomposition, a fluorometry of folic acid can be used as a convenient indicator to evaluate the photosensitizer activities [65, 66]. For example, photosensitized decomposition of folic acid by **Por2** through electron transfer was reported [49]. Photoexcited porphyrin can produce $^1O_2$, and folic acid is also oxidized by $^1O_2$. The contribution of $^1O_2$-mediated decomposition can be excluded by the effect of $^1O_2$ quencher and the effect of electron transfer reaction can be evaluated.

4. Contribution of the electron transfer mechanism in photosensitized reaction by cationic porphyrins

Photooxidation activity through electron transfer depends on the redox potential. It has been demonstrated that photoexcited hematoporphyrin, a free base porphyrin, induces the oxidative electron transfer from the tryptophan residue of bovine serum albumin [67, 68]. Cationic porphyrins show relatively small E_{red} values due to their positive charge. In this section, several examples of electron transfer-mediated oxidation of biomolecules by cationic porphyrins.

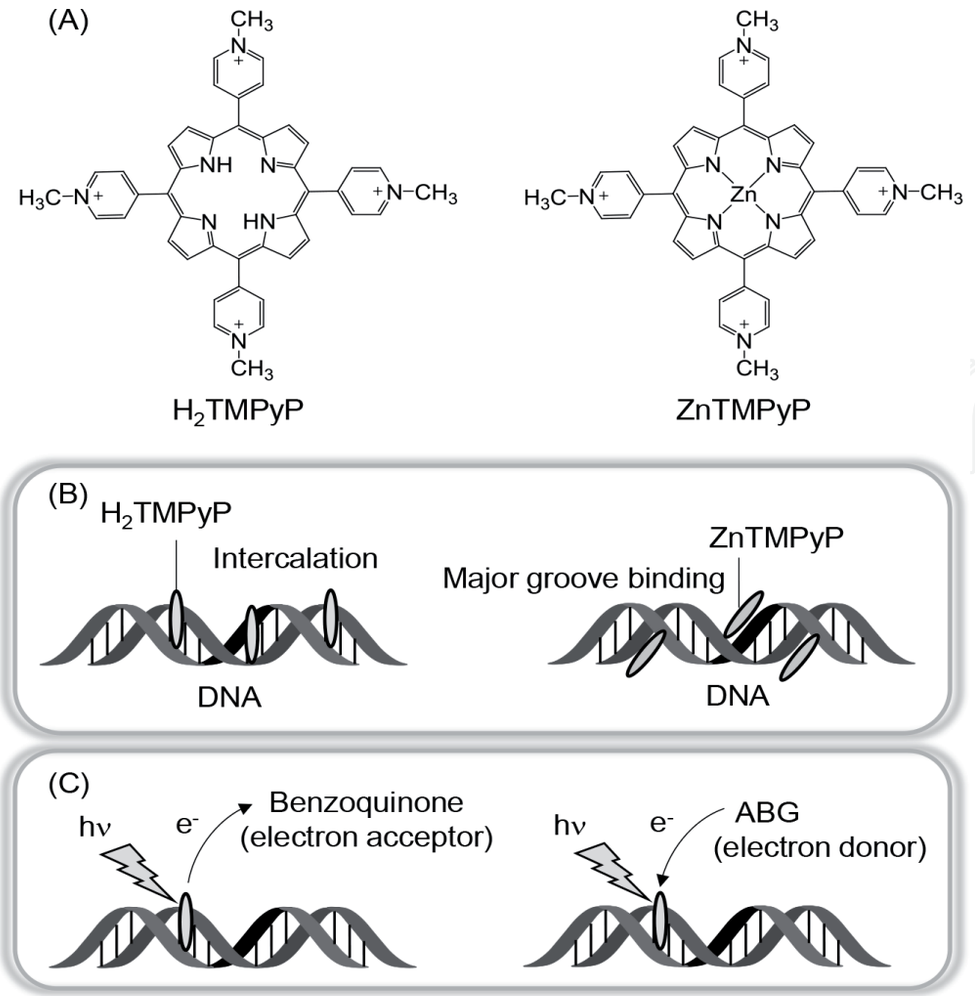


Figure 6.
Structures of H_2TMPyP and ZnTMPyP (A), their binding interaction with DNA (B), and the electron transfer reactions (C). ABG: Amino benzoyl-L-glutamic acid.

4.1 Protein photooxidation through electron transfer by cationic porphyrins

The photosensitized protein damage by tetrakis(*N*-methyl-*p*-pyridinio) porphyrin (H_2TMPyP, **Figure 6**) and its zinc complex (ZnTMPyP, **Figure 6**) was reported [69]. Photosensitized reaction of H_2TMPyP has been extensively studied [14, 70]. Water-solubility of H_2TMPyP and its analogues is appropriate for biological study. Furthermore, electrostatic interaction between these cationic porphyrins and biomacromolecules is considered to enhance the electron transfer reaction with targeting biomolecules. The Φ_Δ value of H_2TMPyP is relatively large [14, 69, 71], and photosensitized biomolecule damage caused by H_2TMPyP through $^1O_2$ generation is generally accepted [70, 72]. However, E_{red} of H_2TMPyP is relatively small [27], and negative ΔG values for photosensitized oxidation of several amino acids through electron transfer are estimated. Therefore, electron transfer-mediated photooxidation of biomolecules is expected.

H_2TMPyP and ZnTMPyP bound to HSA and caused photosensitized oxidation of the tryptophan residue [69]. Three amino acids–tryptophan, phenylalanine, and tyrosine–were also used as target biomolecules, and tryptophan and tyrosine were photodamaged by these cationic porphyrins. However, H_2TMPyP and ZnTMPyP could not photosensitize the damage of phenylalanine. The protein damage (oxidation of the tryptophan residue) was enhanced in deuterium oxide and inhibited by NaN_3. Analysis of the scavenger effect showed that the absolute quantum yields of electron transfer-mediated oxidation are 5.3×10^{-3} and 4.0×10^{-3} for H_2TMPyP

and ZnTMPyP, respectively. The E_{red} of H_2TMPyP (−0.23 V vs. SCE) [27] is lower than that of ZnTMPyP (−0.85 V) [73]. The values of -Δ*G* for electron transfer from tryptophan to their S_1 states suggest that H_2TMPyP (−1.03 eV) is more oxidative than ZnTMPyP (−0.53 eV). The estimated value of k_{ET} estimated from the fluorescence lifetime for H_2TMPyP was 1.0×10^8 s^{-1}. On the other hand, the fluorescence lifetime of ZnTMPyP was not affected by the interaction with HSA in the presented experimental condition. Because of the relatively shorter fluorescence lifetime of ZnTMPyP (1.3 ns), the estimation of k_{ET} may be difficult by the fluorescence lifetime measurement. Furthermore, protein photodamage by the T_1 states of H_2TMPyP and ZnTMPyP were also discussed [69]. The lifetimes of their T_1 states are relatively long: H_2TMPyP (2.1 μs) and ZnTMPyP (2.7 μs), suggesting that the electron transfer in the T_1 state is kinetically advantageous. The estimated -ΔG of the electron transfer from tryptophan to their T_1 states (−0.65 eV for H_2TMPyP and − 0.15 eV for ZnTMPyP) suggests that this electron transfer is also possible in terms of energy.

4.2 Electron transfer from DNA to photoexcited cationic porphyrins and microenvironmental effect of DNA on photoinduced electron transfer

Photoinduced electron transfer between DNA and the cationic porphyrins, H_2TMPyP and ZnTMPyP, was analyzed by the fluorescence measurements (**Figure 6**) [74]. Absorption spectrum and circular dichroism measurements showed that H_2TMPyP mainly intercalates to calf thymus DNA, whereas ZnTMPyP binds into a DNA groove. An electrostatic interaction with DNA raises their redox potentials of the binding cationic porphyrins. In the presence of DNA, the fluorescence intensity of these porphyrins was almost the same as that without DNA. The E_{ox} of H_2TMPyP (>1.30 V vs. SCE in water) [27], ZnTMPyP (1.18 V vs. SCE in water) [73], and guanine (1.24 V vs. SCE in acetonitrile) [75, 76] suggested that electron transfer by the S_1 state of H_2TMPyP is possible in terms of energy. Furthermore, the electron donating character of guanines increased in the double-stranded structure [77–79]. However, the fluorescence measurements indicated that the S_1 states of these porphyrins are barely quenched by DNA. These results could be explained by that an electrostatic interaction between cationic porphyrins and an anionic DNA strand should increase the redox potential of porphyrins, leading to the inhibition of the electron transfer. In the cases of their higher excited states, secondary excited singlet (S_2) states, the electron transfer from DNA was observed. The lifetime of S_2 state is significantly short (a few picoseconds). However, the E_{red} value of their S_2 states are large (larger E_{red} value of the excited state indicates stronger oxidative activity); >2.14 V vs. SCE for H_2TMPyP and 1.94 V vs. SCE for ZnTMPyP. Therefore, the S_2 states of porphyrins are thermodynamically strong oxidants through electron transfer mechanism.

Photoinduced electron transfer from these porphyrins to benzoquinones, electron acceptors, and that from *N*-(4-aminobenzoyl)-L-glutamic acid (ABG), an electron donor, to these porphyrins were also studied [74]. As mentioned above, the electrostatic interaction with DNA raises the redox potential of cationic porphyrins (*i.e.* decreases the oxidative property of cationic porphyrins). Therefore, the DNA microenvironment inhibited the electron transfer from ABG, an electron-donating quencher, to the binding porphyrins. On the other hand, the electron transfer from the binding porphyrins to benzoquinones, an electron-accepting quencher, was enhanced. A steric effect by the DNA strand was also important. A hydrophobic bulky electron acceptors forms stacking complex with porphyrins, resulting in the strong fluorescence quenching. The interaction with DNA strand cleaves this stacking interaction and inhibit the electron transfer to the benzoquinone. In summary,

the DNA microenvironment significantly affects the electron transfer property of the binding cationic porphyrins through an electrostatic interaction and the steric effect.

5. Activity control based on the electron transfer

Electron transfer can be controlled by the surrounding environment. For example, pH is an important factor to control the photoinduced electron transfer [29, 30, 48, 80, 81]. Since it has been reported that cancer cells are slightly acidic (pH 6 ~ 7) against normal tissues (pH 7 ~ 7.4) [82–85], control of the electron transfer of the photosensitizer by pH can be applied for the development of cancer-selective PDT. In the cases of pH-dependent $^1O_2$ photosensitizers, the redox control [30, 86–88], the structure change [89], and the control of intersystem crossing [90] by pH have been reported as the important concepts. Several types of pH-activatable-porphyrin photosensitizers [30, 88], including a phosphorus(V) porphyrin [48, 81], have been reported. In addition, a self-quenching of the photoexcited molecules can be also used to control the activity [47]. In this section, several examples about the activity control of electron transfer-photosensitizers are introduced.

5.1 Electron transfer control by pH

The biomolecule oxidation activity of photosensitizer through electron transfer can be controlled by using changeable electron donor. **Por13** was designed and synthesized to control the photodynamic activity of phosphorus(V) porphyrin photosensitizer (**Figure 7**) [48]. As an electron-donor, 6-methylpyridine was used. The photoexcited **Por13** is quenched through intramolecular electron transfer and this quenching is suppressed by protonation of the methylpyridine moiety, an electron donor. The pK_a of protonated methylpyridine moiety was about 7, and fluorescence lifetime of **Por13** was lengthened under an acidic condition by

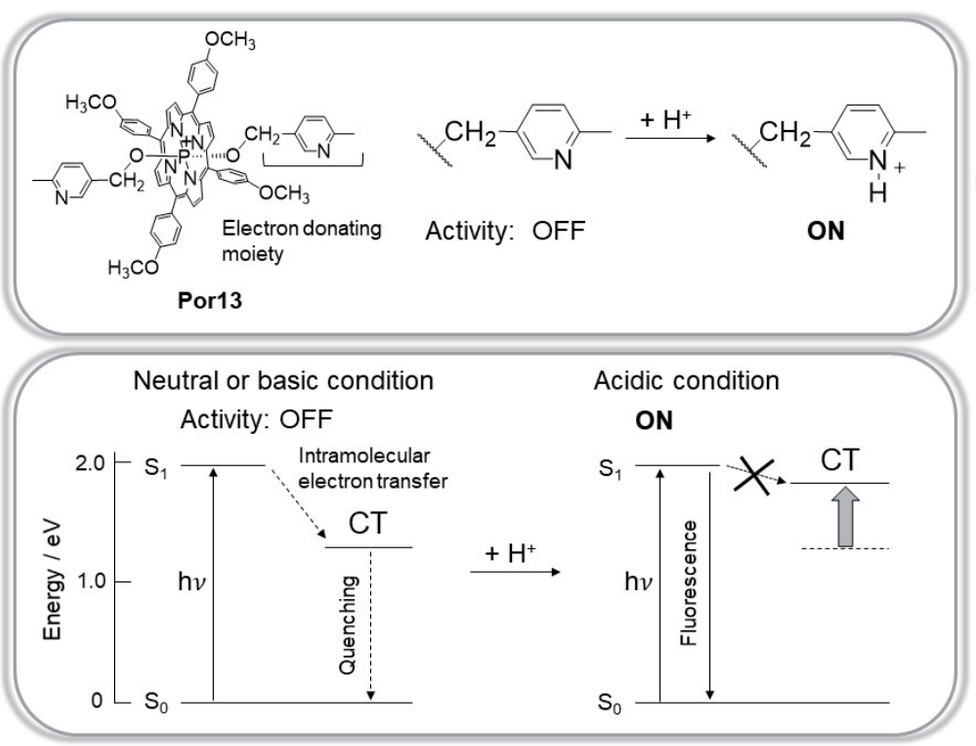


Figure 7.
Scheme of the activity control of photosensitizer, Por13, by pH and the relaxation processes of photoexcited state.

suppression of the quenching through intramolecular electron transfer by methylpyridine. The quantum yields of photosensitized $^1O_2$ generation and biomolecule oxidation through electron transfer mechanism were also increased under acidic condition. NADH oxidation by **Por13** through photoinduced electron transfer was successfully enhanced under acidic conditions. However, photosensitized protein damage (oxidative damage of HSA) through electron transfer was decreased under an acidic condition, and relatively strong protein damage was observed under a neutral condition. It is explained by the fact that a relatively weak association between protein and **Por13** under an acidic condition due to electrostatic repulsion. Protonated protein under acidic condition decreases the association with cationic porphyrin, resulting in the suppression of the electron transfer from the amino acids. Furthermore, the hydrophobic environment of protein inhibits the electron transfer-quenching of **Por13**. This study shows the difficulty of activity control of photosensitizers by pH, because other factors significantly affect the photoinduced electron transfer.

5.2 Activity control through the self-quenching of photosensitizers

DiethoxyP(V)tetrakis(*p*-methoxyphenyl)porphyrins, **Por10** and **Por11**, analogues of above mentioned **Por9**, were synthesized [47]. Their water-solubilities were smaller than that of **Por9**, and these porphyrins form self-aggregation complexes (**Figure 8**). Photoexcited states of **Por10** and **Por11** were effectively quenched through this aggregation (concentration quenching). These phosphorus(V) porphyrins can bind to the hydrophobic pocket of HSA, resulting in dissociation of their self-aggregation states (**Figure 8**). Calculating simulation showed the distance between the tryptophan residue and the porphyrin molecules as follows: 24.4 Å (**Por10**) and 23.5 Å (**Por11**). Fluorescence lifetime of these porphyrins were recovered by the dissociation of self-aggregation. Photoirradiation to these porphyrins binding to HSA induced the oxidation of tryptophan through $^1O_2$ generation and electron transfer. The axial fluorination of ethoxy chain of central phosphorus atom reduced the E_{red} of porphyrin ring. The electron transfer

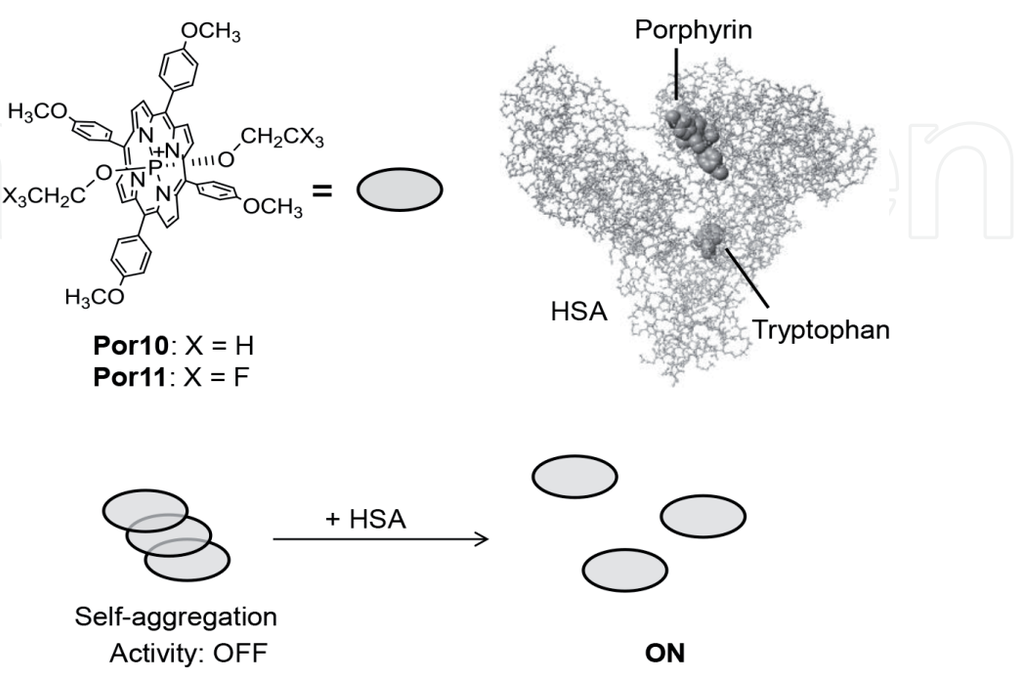


Figure 8.
Scheme of the activity control of photosensitizers, **Por10** *and* **Por11***, through the self-aggregation and interaction with HSA.*

rate constant from the tryptophan residue of HSA to **Por11** is larger than that of **Por10**, due to the effect of axial fluorination. The substitution by fluorine, the highest electronegative element, showed the improving effect on photooxidation of protein through electron transfer. However, the fluorination decreased the binding interaction with HSA. In the presence of same concentration of porphyrins, **Por10** exhibits higher damaging activity to HSA under photoirradiation. These results suggest that selective interaction is important for electron transfer-mediated photodamage of biomolecules. These porphyrins demonstrated the photocytotoxicity to HaCaT cells. The IC50 value of **Por11** was lower (stronger cytotoxicity) that **Por10**. Photooxidative activity of **Por11** through electron transfer and enhanced cellular uptake by the fluorination may play the important role in this photocytotoxic effect. Furthermore, **Por10** and **Por11** barely induce cellular DNA damage to HaCaT cells, similarly to **Por8**, **Por9**, and **Por12**. Therefore, their carcinogenic risks are also small. The self-aggregation of photosensitizers can be used to suppress their photosensitizing activity. These results suggest that the PDT activity of self-aggregation photosensitizers can be reversed using association with targeting biomacromolecules, such as protein.

6. Electron transfer mechanism and antimicrobial photodynamic therapy

PDT can be applied for disinfection and sterilization [4–7]. Microbial, including bacterium and viruses can be removed by photosensitized reaction. The physical treatment, such as PDT, is advantageous against antibiotic-resistant bacteria [91, 92]. PDT for microbial treatment is called as aPDT and/or PACT. Red light (relatively long wavelength visible light) is used for aPDT. Because $^1O_2$ can be easily produced by relatively small energy photons, it is considered as the important reactive species for aPDT process. Phenothiazine dyes, such as Methylene Blue is used as the photosensitizer for aPDT [93], because Methylene Blue can absorb relatively long-wavelength visible light and its Φ_Δ value is relatively large [94]. However, the aPDT mechanism has not been well-understand. Biological environments are under a hypoxic condition [95], the mechanism mediated by $^1O_2$ generation mechanism may be restricted. Therefore, the electron transfer mechanism may play an important role in the aPDT mechanism.

6.1 Photosensitized DNA damage through electron transfer

DNA is a potentially important targeting biomacromolecules for PDT and aPDT [1–3, 28]. In the cases of DNA damage, the generation of reactive oxygen species, such as $^1O_2$ (Type II mechanism), and the direct oxidation of nucleobases through photoinduced electron transfer (Type I mechanism) are important. In general, $O_2^{\bullet-}$ formation and following H_2O_2 and/or $^\bullet OH$ production (Type II mechanism, minor) require relatively shorter wavelength radiation, such as ultraviolet ray [28, 32, 33]. Therefore, the contribution of the $O_2^{\bullet-}$ generation (Type II minor) mechanism is considered to be small in the aPDT mechanism. As mentioned above, photosensitized $^1O_2$ generation is the important mechanism of aPDT. Guanine is the selective target of $^1O_2$, and every guanine is oxidized by $^1O_2$ in a DNA sequence [28, 33]. Similar to the $^1O_2$ generation mechanism, guanine is also damaged through electron transfer selectively [28, 32, 33]. However, single guanines in double-stranded DNA and guanine residue in single-stranded DNA are resistant to electron transfer mechanism, in the contrary to the $^1O_2$ mechanism [28, 33]. Since π-π interaction between consecutive guanines decrease the E_{ox} of guanine, the consecutive

guanines, such as GG and GGG, are selectively oxidized through electron transfer mechanism [77–79]. Similar compounds are produced of guanine oxidation through the both mechanisms of $^1O_2$ generation and electron transfer [72].

The mechanism of DNA damage photosensitized by Nile Blue (**Figure 9**) has been studied as a potential photosensitizing reaction [96]. The reported value of Φ_Δ by Nile Blue is very small (0.005) [66, 97]. Therefore, Nile Blue is an appropriate model to examine the oxygen-independent mechanism. Nile Blue bound to DNA strand through an electrostatic interaction and the fluorescence lifetime was decreased, supporting the electron transfer quenching. Using ^{32}P-5′-end-labeled DNA fragments, DNA damaging mechanism of Nile Blue was examined and consecutive guanine damage was observed. From the analysis of DNA damaging pattern, the contribution of DNA damage through electron transfer mechanism was estimated to be 72% (the contribution of $^1O_2$ mechanism is 28%). The ΔG of electron transfer from guanine to the S_1 state of Nile Blue is negative (−0.15 eV) [96], and this value is considered to become smaller in the case of consecutive guanine, as mentioned above [77–79]. The estimated k_{ET} value is relatively large (1.0×10^{10} s^{-1}). These values supported the electron transfer-mediated DNA oxidation. The mechanism of DNA damage photosensitized by Nile Blue is shown in **Figure 9**. Relevantly, rhodamine-6G, a fluorescence dye, induces the electron transfer-mediated oxidation of DNA [98] and folic acid [64] with photoirradiation. In general, fluorescence dyes hardly photosensitize $^1O_2$ generation. On the other hand, photooxidative activity through electron transfer depends on the redox potential of molecules. These results suggest that the electron transfer-oxidation becomes important PDT mechanism for non-$^1O_2$ generating dyes.

6.2 Photosensitized protein damage through electron transfer

Photosensitized protein damage by Methylene Blue and its analogues (**Figure 10**) were studied [99]. Similar to the cases of phosphorus(V) porphyrin photosensitizers, HSA was used as the targeting biomacromolecules. DNA binding through electrostatic force of these cationic compounds are well-known [40, 71, 74, 96, 100]. However, the interaction between these cationic dyes and HSA is small and a hydrophobic

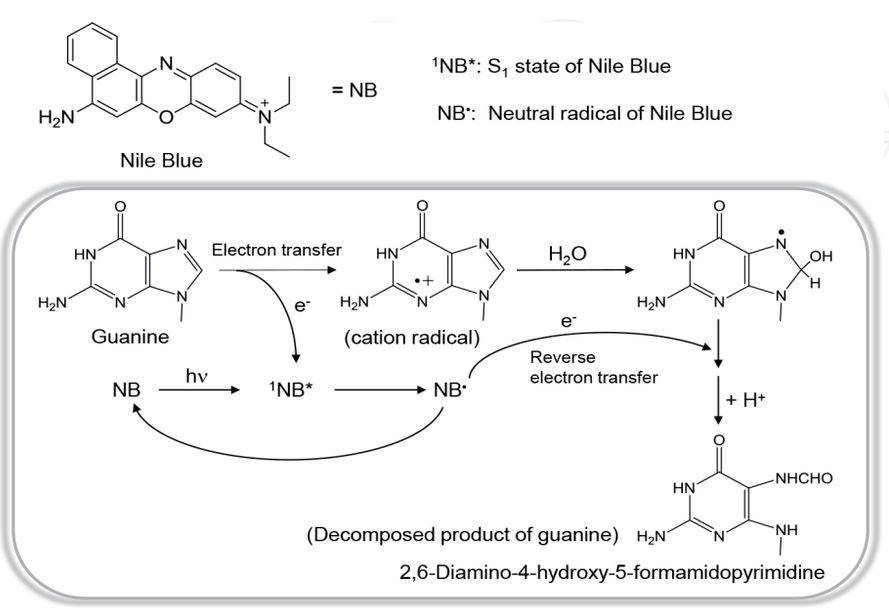


Figure 9.
Structure of Nile Blue and the proposed mechanism of guanine decomposition through photoinduced electron transfer.

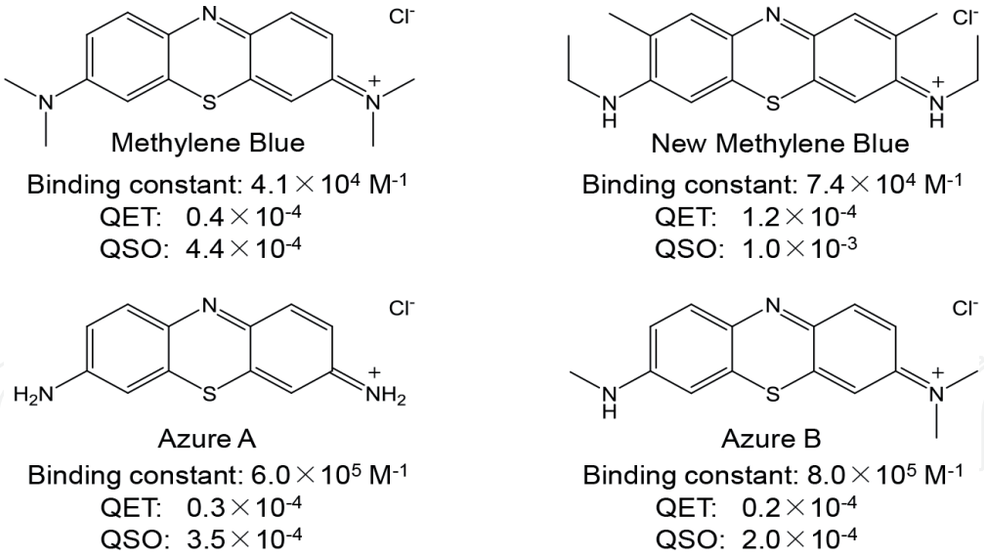


Figure 10.
Structures of Methylene Blue and its analogues. Binding constants with HSA were examined in a 10 mM sodium phosphate buffer (pH 7.6). QET: The quantum yield of HSA oxidation through electron transfer mechanism. QSO: The quantum yield of HSA oxidation through $^1O_2$ generation.

interaction (not electrostatic interaction) may be a driving force of the association with HSA [58]. The reported binding constant, which were estimated by the Benesi-Hildebrand Equation [101] are shown in **Figure 10**. Fluorometry of HSA tryptophan residue demonstrated the photosensitized oxidation through both mechanisms, electron transfer and $^1O_2$ generation [99]. The analyzed quantum yields through these mechanisms are shown in **Figure 10**. Fluorescence decay of these dyes was complex. From the analysis of their observed fluorescence decay, the estimated k_{ET} values were order of 10^9 s^{-1}, supporting the electron transfer mechanism. Furthermore, this result suggests the existence of markedly fast electron transfer species, much faster than the detection limit of this study (within ~50 ps) [99]. DFT calculation also supported the electron transfer mechanism. The energy gap between the highest occupied molecular orbital (HOMO) of amino acids and that of photosensitizers are important for the electron transfer mechanism. The plot between the HOMO values of these cationic dyes and the protein damaging quantum yield through electron transfer demonstrated a relatively good relationship. Furthermore, the relationship between the Φ_Δ and the damaging quantum yield through $^1O_2$ generation is also observed. These results shown that the electron transfer mechanism is also important for photosensitized protein oxidation by Methylene Blue and its analogues, as $^1O_2$ generation mechanism does. The electron transfer mechanism is not completely independent of oxygen molecule, because oxygen support the electron transfer by removing the excess electron from the reduced photosensitizer. However, other endogenous oxidative agents, such as metal ions, may support the electron transfer mechanism, in *vivo*, the electron transfer mechanism may play an important role in the aPDT under hypoxic condition.

7. Conclusions

This chapter reviewed the several topics about the photosensitizers, which play electron transfer-supported mechanism. $^1O_2$ is the important reactive species in PDT and aPDT. However, hypoxic condition in biological environment is not appropriate for reactive oxygen-dependent mechanism. Electron transfer is not completely independent of oxygen; however, this mechanism does not absolutely require oxygen. Endogenous oxidative substances other than oxygen can support the electron

transfer mechanism. In the study of PDT photosensitizer for cancer, phosphorus(V) porphyrins showed the selectivity for cancer cell and relatively strong PDT effects. Most important property of these photosensitizers is strong photooxidative activity through electron transfer under long-wavelength visible light irradiation. Furthermore, the photosensitizing activity of phosphorus(V) porphyrins through electron transfer mechanism can be controlled by surroundings, such as pH. In the processes of aPDT, the electron transfer mechanism may be important. For developing the effective drugs for aPDT, molecular design based on the electron transfer is also useful as well as that based on the $^1O_2$ generating activity. The activity of electron transfer oxidation depends on the redox potential, and a long lifetime of photoexcited state is advantageous. For PDT photosensitizers, relatively strong response to long-wavelength radiation is required. In the molecular design of PDT photosensitizers including phosphorus(V) porphyrins, the calculations of HOMO energy level and the excitation energy are important as the initial steps.

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Conflict of interest

The author declares no conflict of interest.

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