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Ligand release from silicon phthalocyanine (SiPc) dyes by near infrared (NIR) light is a key photochemical reaction for caged compounds based on SiPc. Although NIR light is relatively permeable compared with visible light, light can be attenuated by tissue absorption and scattering; therefore, using light to induce a photochemical reaction deep inside the body is difficult. Herein, because X-rays are highly permeable and can produce radicals by radiolysis of water, we investigated whether the axial ligands of SiPcs can be cleaved by X-ray irradiation. SiPcs with different axial ligands (alkoxy, siloxy, oxycarbonyl and phenoxy groups) were irradiated with X-rays under hypoxic conditions. We found that the axial ligands were cleaved via a reaction with hydrated electrons (e^-_{aq}), not OH radicals, generated from water by X-ray irradiation and that SiPc with alkoxy groups exhibited the highest cleavage efficiency. A quantitative investigation revealed that the X-ray-induced axial ligand cleavage proceeds via a radical chain reaction. The reaction is expected to be applicable to molecular design for X-ray-activatable functional molecules in the future.

Introduction

Activatable functional molecules by specific conditions are widely used as a useful tool in basic research and expected to be applied in human use.[1,2] Among them, caged compounds are molecules that can release drugs or biomolecules by external stimuli, such as light.[3] Since light irradiation is spatiotemporally controllable, the caged compounds can be used as prodrugs activated only at the irradiated sites, reducing the systemic side effects. However, since UV to visible light is usually used for the activation and has low tissue permeability, the development of caged compounds that work in living organisms is still challenging. Therefore, most of the application has been limited to in vitro experiments.[4-6] Recently, caged compounds in response to near infrared (NIR) light have been developed, which are applicable to mice due to relatively high permeability compared to UV to visible light.[7-10] However, since the permeability is still insufficient for living organisms, the application in deep tissues is difficult. On the other hand, among electromagnetic waves, X-rays in the wavelength of 0.001-0.1 nm (so called hard X-rays) are very attractive as an external stimulus to change the structure of compounds due to the high tissue permeability and widespread availability of the X-ray generator in clinic. However, the energy of X-rays is much higher than that of excitation of small molecules, making structural changes difficult based on the mechanism of the reported compounds. Therefore, as another approach, we considered using various reactive chemical species produced by the radiolysis of water (*e.g.* hydrated electrons (e^-_{aq}), hydrogen radicals, and OH radicals) for changing in structure of compounds by X-ray irradiation.[11]

Recently, we have elucidated the mechanism of light-induced axial ligand cleavage of silicon phthalocyanines (SiPcs) (Fig. 1a).[12,13] The photocleavage can be used for the molecular design of caged compounds.[8,14] The mechanism is as follows (Fig. 1b): Upon absorbing NIR light, SiPcs are excited to the singlet state and transitions to the triplet sate via intersystem crossing (ISC), although the quantum yield of ISC is not high (Φ_{ISC} = 0.019).[15] Then, the SiPcs in the triplet excited state receive an electron from an electron donor to produce an anion radical, and the axial ligand of the anion radical is protonated by hydronium ion. Axial ligand exchange occurs, including cleavage of the axial ligands and coordination of a hydroxy ion. We found that in this reaction the axial ligand cleavage proceeds spontaneously after the formation of anion radicals at room temperature.[13] Therefore, if anion radicals of SiPcs are generated by the reaction with the reactive species derived from water radiolysis, X-ray irradiation may induce the axial ligand cleavage of SiPcs. Using this mechanism, it is possible to develop X-ray activatable caged compounds. Herein, we investigated whether axial ligand cleavage could be induced by X-ray irradiation to SiPcs derivatives.

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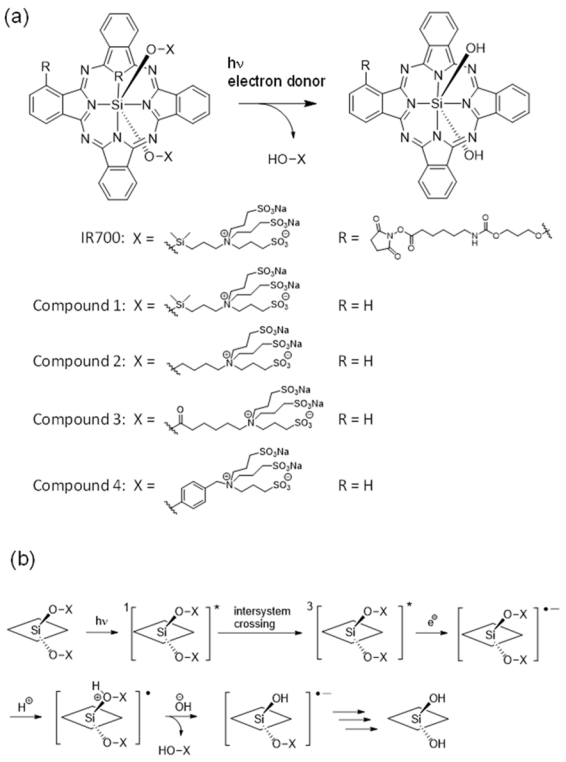


Fig. 1. Photoinduced axial ligand cleavage of silicon phthalocyanines. (a) The cleavage reaction of the axial ligand of IR700 and compounds **1**-**4** by near-infrared (NIR) light. The cleavage is induced by NIR light in the presence of an electron donor, and the hydrophobic compound was generated because of the loss of hydrophilic axial ligands. (b) Plausible mechanism of axial ligand cleavage of SiPcs. Upon absorption of excitation light, the compounds are excited to the singlet state and then transition to the triplet state, leading to the generation of anion radicals by electrons received from an electron donor. Next, stepwise axial ligand exchange, including protonation, cleavage, and the incorporation of a hydroxy ion, occurs spontaneously to produce SiPc compounds with a uniaxial ligand. By repetition of the reaction, hydrophobic SiPc compounds with dihydroxy groups are finally obtained.

Results and discussion

Axial ligand cleavage of SiPcs triggered by X-ray irradiation

We have previously developed SiPcs with different axial ligands (compounds **1**-**4**, Fig. 1a) and investigated the axial ligand cleavage by NIR light in the presence of an electron donor.[16] In the present work, we first examined whether axial ligand cleavage of compounds **1**-**4** could be induced by X-ray irradiation using a linear accelerator (LINAC). Aqueous 5 μM solutions of compounds **1**-**4** were irradiated with X-rays at 20 Gy under hypoxic conditions, and the solutions were subsequently analyzed by high-performance liquid chromatography (HPLC) using methylene blue (MB) as an internal standard. After X-ray irradiation, the peaks of compounds **1**-**4** almost disappeared and peaks of the degraded products were observed in the chromatograms, which were identical to those of SiPc with one or both of the axial ligands cleaved (Fig. 2a-d). However, when oxygen was present, the X-ray-induced axial ligand cleavage was inhibited (Fig. 2e). These results suggest that radicals that can be quenched by oxygen were involved in the axial ligand cleavage reaction. We further analyzed the cleavage reaction to identify the radicals.

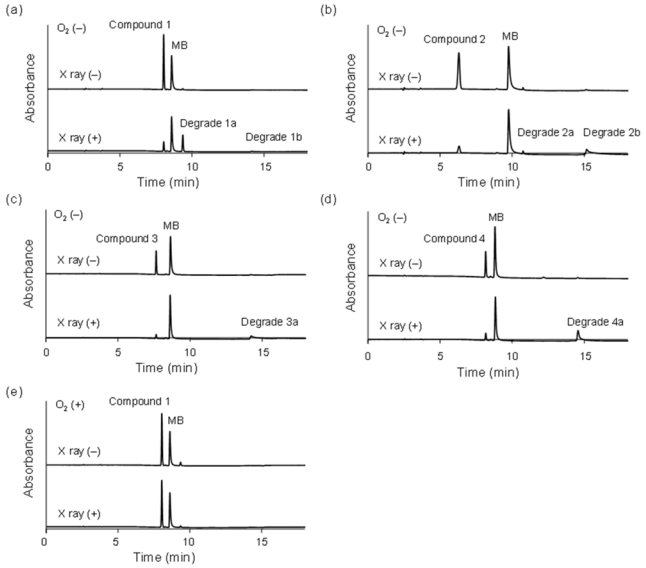


Fig. 2. HPLC chromatograms of compounds **1**-**4** after X-ray irradiation. 5 μM solutions of compounds **1**-**4** were irradiated with X-rays at 20 Gy in the presence or absence of oxygen. The solution with methylene blue (MB) as an internal standard was analyzed by HPLC. (a) Compound **1**, (b) compound **2**, (c) compound **3** and (d) compound **4** irradiated in the absence of oxygen. (e) Compound **1** irradiated in the presence of oxygen.

Radicals involved in the axial ligand cleavage of SiPc by X-ray irradiation

We next investigated the radical involved in the X-ray-induced axial ligand cleavage. As described in the Introduction, water radiolysis results in the generation of mainly OH radicals and e^-_{aq} whose G-values are 2.7 and 2.6, respectively (the G-value indicates the number of molecules formed by the absorption of 100 eV energy in the system).[17] To investigate the contribution of these radicals to the X-ray-induced axial ligand cleavage, OH radicals and e^-_{aq} were scavenged by excess sodium formate and N_2O, respectively. Eventually, OH radicals and e^-_{aq} were converted into CO_2 anion radicals and OH radicals, respectively (eq. 1, 2):

$$^{\bullet}OH + HCO_2^- \longrightarrow H_2O + CO_2^{-\bullet} \quad (1)$$

$$e^-_{aq} + N_2O + H_2O \longrightarrow {}^{\bullet}OH + {}^-OH + N_2 \quad (2)$$

In the presence of sodium formate (condition II), e^-_{aq} and CO_2 anion radicals exist, whereas in the presence of N_2O (condition III), only OH radicals exist in the solutions. In the case of the addition of both sodium formate and N_2O (condition IV), CO_2 anion radicals exist in abundance (Fig. 3a).

To evaluate the involvement of the radicals in the X-ray-induced axial ligand cleavage, in addition to HPLC analysis, electron spin resonance (ESR) spectroscopy was also performed. ESR spectroscopy is usually conducted using high-concentration samples. Thus, we performed the experiment using 500 μM solutions of compounds **1**-**4** and high-dose X-ray irradiation (210 Gy) under hypoxic conditions. A high-dose X-ray generator (X-Rad iR-225) was used for X-ray irradiation within a short time

(dose rate: 210 Gy/min). In a preliminary experiment, we realized that the cleavage efficiency could change depending on when the hypoxic samples were aerated after X-ray irradiation. To investigate the influence of the timing of aeration, we compared the following two conditions: aeration immediately after X-ray irradiation and aeration 30 min after X-ray irradiation. Given that the decrease in concentration of compounds **1**-**4** indicates the progress of the axial ligand cleavage, we compared the remaining percentage of compounds **1**-**4** after X-ray irradiation, as calculated using the internal standard method with MB. The results show that little axial ligand cleavage occurred in compounds **1**-**4** under conditions I (no radical scavengers) and III; however, under conditions II and IV, the axial ligands of compounds **1** and **2** were cleaved for both aerations immediately and 30 min after X-ray irradiation (Fig. 3b). In addition, the axial ligand cleavages of compounds **1** and **3** were enhanced when hypoxia was maintained for 30 min under conditions II and IV compared with the axial ligand cleavages under immediate aeration. For compound **2**, the axial ligands were completely cleaved under immediate aeration; we therefore could not confirm the cleavage enhancement effect of compound **2** when hypoxic conditions were maintained. These results suggest that e^-_{aq} or CO_2 anion radicals were involved in the axial ligand cleavage reaction and that the cleavage reaction proceeded for a few tens of minutes.

To investigate the generation of SiPc radicals, ESR measurements were carried out after irradiation of 210 Gy X-rays under condition IV. As a result, long-lived ESR signals with a broad linewidth were observed; these spectral features are the same as those observed for the anion radical of compound **1** generated when compound **1** was irradiated with NIR light in the presence of electron donors (Fig. 3c).[18] In addition, the signals quickly disappeared upon aeration, as was observed for the anion radical of compound **1**. These results suggest that the anion radicals of compounds **1**-**4** were generated by X-ray irradiation under reductive conditions. In addition, after X-ray irradiation, the precipitate was produced in the solution, suggesting that the axial ligand was cleaved to produce the insoluble degradation compound, $SiPc(OH)_2$ (Fig. 3d).

Next, to further investigate the involvement of e^-_{aq} or CO_2 anion radicals, we examined whether the dose rate affects the axial ligand cleavage. The reaction rate depends on the concentration (amount) of radicals generated per unit time because of the short lifetimes of the e^-_{aq} and CO_2 anion radicals. In addition, if e^-_{aq} or CO_2 anion radicals are involved in the reaction, then the efficiency of the cleavage reaction should be affected by the dose rate. Thus, we investigated the cleavage of the axial ligand of compound **2** when irradiated with 50 Gy X-rays at different dose rates (4.7, 58, and 210 Gy/min). The results show that the axial ligand was cleaved at 210 Gy/min but not at 4.7 or 58 Gy/min (Fig. 3e), indicating that e^-_{aq} or CO_2 anion radicals with short lifetimes are involved in the reaction, as expected.

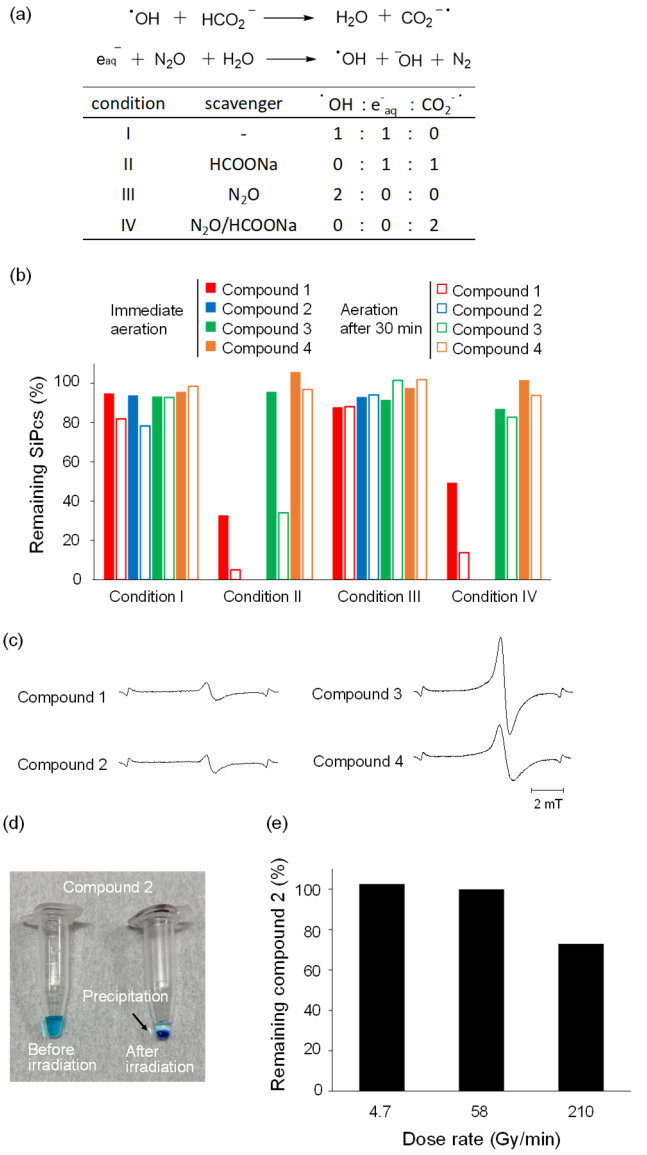


Fig. 3. X-ray-induced axial ligand cleavage in the presence of radical scavengers. (a) The ratio of radicals generated by water radiolysis in the presence of radical scavengers, such as sodium formate (HCOONa) and N_2O. (b) Comparison of the axial ligand cleavage of compounds **1**-**4** when aerated immediately after X-ray irradiation or 30 min after X-ray irradiation under conditions I-IV. 500 μM solutions of compounds **1**-**4** were irradiated with X-rays at 210 Gy (equivalent to ~120 μM reactive species generated by X-rays) in the presence of HCOONa or N_2O under hypoxic conditions. The solution with MB as an internal standard was analyzed by HPLC. (c) ESR signal of compounds **1**-**4** after X-ray irradiation under condition IV. (d) Photograph of a sample of compound **2** in a tube before and after X-ray irradiation. The precipitate was produced after X-ray irradiation. (e) Dose-rate effect of the axial ligand cleavage of compound **2**. A 500 μM solution of compound **2** was irradiated with X-rays at 50 Gy (equivalent to ~28 μM reducing species) under condition II, and the hypoxic condition in the solution was maintained for 30 min.

Radical chain reaction induced by X-ray irradiation

Because we speculated that the efficiency of the X-ray-induced axial ligand cleavage was high, we also quantitatively evaluated the radical cleavage reaction of axial ligands. On the basis of the G-values, a total ~120 μM of OH radicals and e^-_{aq} were generated by X-ray irradiation by water radiolysis at 210 Gy. Therefore, under conditions II and IV, 120 μM of reducing

species (e^-_{aq} or CO_2 anion radicals) were produced, which is equivalent to ~25% of 500 μM compounds **1**-**4**. Surprisingly, however, under conditions II and IV, almost all of compound **2** disappeared after X-ray irradiation under both aeration conditions. In addition, the concentration of compound **1** decreased by more than 50% after immediate aeration and more than 90% after aeration 30 min after X-ray irradiation. These results suggest that the amount of compounds **1** and **2** decreased by more than the amount of reducing species generated by water radiolysis (Fig. 3b). Thus, the results strongly suggest that the axial ligands could be cleaved via a radical chain reaction involving reducing species, such as e^-_{aq} or CO_2 anion radicals.

The axial ligand of compound **2** was completely cleaved under conditions II and IV in Fig. 2b. Thus, further investigation was carried out using compound **2** to determine the lower limit of the X-ray irradiation dose for complete cleavage when the hypoxic condition was maintained for 30 min under conditions II and IV. As a result, under condition II, the threshold of the dose was 25-50 Gy; by contrast, under condition IV, the threshold was greater than 100 Gy (Fig. 4a).

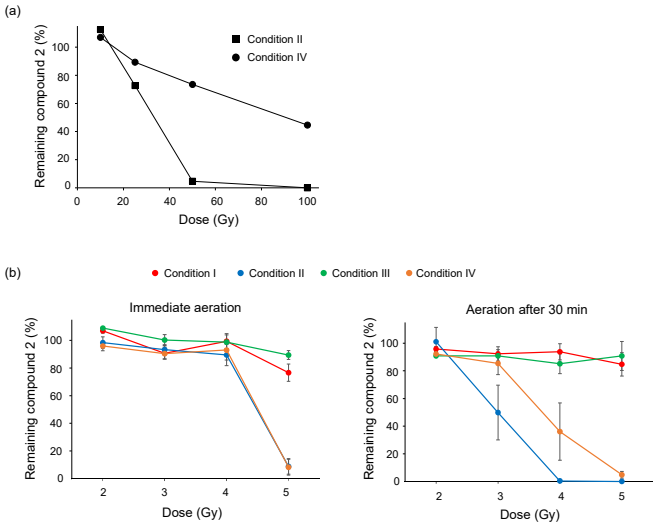


Fig. 4. Determination of the lower limit of X-ray radiation dose for complete cleavage of compound **2**. (a) Comparison of the axial ligand cleavage of 500 μM compound **2** under conditions II and IV at lower doses (10-100 Gy). (b) The axial ligand cleavage of compound **2** at lower doses; a 5 μM solution of compound **2** was irradiated with X-rays at 1-5 Gy (equivalent to 0.55-2.8 μM reducing species) in the presence of HCOONa or N_2O under conditions I-IV. The left and right figures show a comparison of a sample aerated immediately after X-ray irradiation and one aerated 30 min after X-ray irradiation, respectively.

We also investigated whether the axial ligand of compound **2** could be cleaved at a lower concentration of compound **2** irradiated with lower X-ray doses. A 5 μM solution of compound **2** was X-ray-irradiated with LINAC at 1-5 Gy (equivalent to 0.55-2.8 μM reactive species) under conditions I-IV. The results show that the response to X-rays under these conditions was similar to that in the case of higher radiation doses (Fig. 4b). No axial ligand cleavage was observed under conditions I and III, whereas the axial ligand was cleaved under conditions II and IV. When the solution was aerated 30 min after irradiation under condition II, the threshold of the dose for complete cleavage was between 3 and 4 Gy, showing that the axial ligands were cleaved more than the amount of reducing species generated by water radiolysis (2.2 μM reducing species were generated by X-ray irradiation at 4 Gy). Thus, the results show that the radical chain reaction occurred even at low concentrations with lower radiation doses, and that a 5 μM sample of compound **2** completely disappeared upon 4 Gy irradiation.

Reaction mechanism of X-ray-induced axial ligand cleavage

Next, we investigated the reaction mechanism of the X-ray-induced axial ligand cleavage. We compared the amount of anion radicals generated in the radical chain reaction and the extent of axial ligand cleavage. A 500 μM solution of compound **2** was irradiated with 50 Gy X-rays under condition II, and the concentration of compound **2** and the amount of anion radicals were measured over time after X-ray irradiation. A calibration curve for the ESR measurement was prepared using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a standard sample. Immediately after X-ray irradiation, approximately 28 μM of anion radicals of compound **2** was produced, which is approximately the same concentration of the reducing species generated at 50 Gy irradiation; the concentration of anion radicals then decreased rapidly in 5-15 min (Fig. 5a). The HPLC analysis results also show that the amount of compound **2** decreased rapidly in 5-15 min (Fig. 5a). Comparing these results, we find that both the concentrations of anion radicals and compound **2** decreased almost in parallel, suggesting that anion radicals are strongly involved in the radical chain reaction following axial ligand cleavage (Fig. 5b).

We next examined the position of the bond cleavage in the radical chain reaction because there were two candidates for the cleavage position (*i.e.*, the Si-O and the O-CH_2R bond in the structure of Si-O-CH_2R). For this purpose, compound **2** was irradiated with X-rays in [^{18}O]H_2O and the degradation products were analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). If the Si-O bond is cleaved, the degraded compound $SiPc(OH)_2$ should include an [^{18}O]hydroxy group derived from [^{18}O]H_2O. The mass spectrum shows a fragment peak of $SiPc(OH)_2$ containing an [^{18}O]hydroxy group, which suggests that axial ligand cleavage occurred at the Si-O bond of compound **2** and that a hydroxy ion derived from water coordinated to the intermediate (Fig. 5c).

We next investigated the pH-dependency of the X-ray-induced axial ligand cleavage. Because the photoinduced axial ligand cleavage proceeds via protonation of the anion radicals, the cleavage efficiency is affected by the pH of the solution. That is, the efficiency increases with decreasing pH. We therefore investigated whether X-ray-induced axial ligand cleavage is also affected by pH. When compound **2** was irradiated with X-rays at pH 6 and 7, the cleavage efficiencies of the axial ligand were found to be higher at pH 6, suggesting that the axial ligand cleavage occurred through the same reaction as the photochemical reaction (Fig 5d).

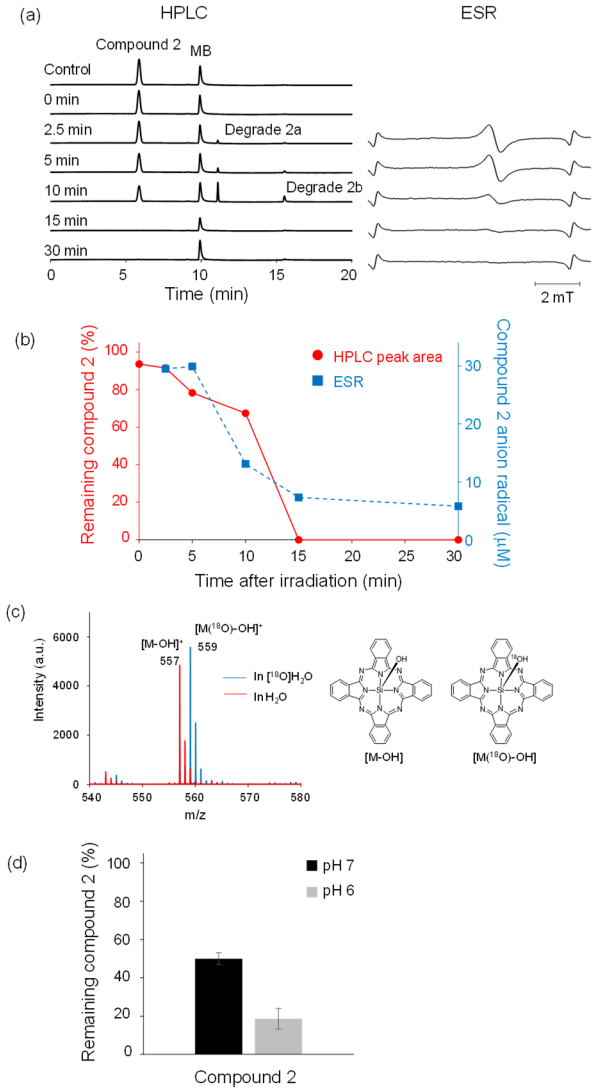
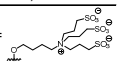


Fig. 5. Investigation of the mechanism of the radical chain reaction of compound **2** triggered by X-ray irradiation. (a) HPLC chromatogram of compound **2** irradiated with X-rays and its ESR signal over time. The time after X-ray irradiation is shown. (b) The correlation between the amount of anion radical and the amount of cleaved axial ligand. A 500 μM solution of compound **2** was irradiated with X-rays at 50 Gy (equivalent to ~28 μM reducing species) under condition II and the hypoxic condition in the solution was maintained at each timepoint. (c) MALDI-TOF MS spectra of compound **2** irradiated with X-rays under hypoxic conditions in H_2O or $[^{18}O]H_2O$. The plausible chemical structures of the degraded products are shown. (d) The axial ligand cleavage of compound **2** at pH 6 and 7. A 5 μM solution of compound **2** was irradiated with X-rays at 20 Gy under condition I.

Computational studies on the radical chain reaction and electron attachment

Quantum chemical calculations were conducted to examine whether the radical chain reaction of SiPcs occurs. The SiPc anion radicals are involved in the axial ligand cleavage and have a very long lifetime. The radicals are therefore considered an important intermediate in the radical chain reaction. In a previous investigation, the anion radical was found to still exist after axial ligand exchange in the cleavage reaction (Fig. 1b, $[Si(OX)_2]^{\bullet-} + H_2O \longrightarrow [Si(OX)(OH)]^{\bullet-} + XOH$).[18] Therefore, if the anion radical reacts with SiPcs in the ground state after ligand exchange, the radical chain reaction can proceed. We optimized the geometry of compounds **1** and **2** [$SiPc(OX)_2$], their degradation products (*i.e.*, $SiPc(OH)(OX)$ and $SiPc(OH)_2$), and their anion radical forms to evaluate whether the electron transfer reaction from a degraded anion to an undegraded neutral species occurs spontaneously. Table 1 summarizes the change in Gibbs energy (ΔG) for each conceivable electron transfer reaction. For all of the electron transfer reactions to undegraded species and both for compounds **1** and **2**, the ΔG values are negative, indicating that the reactions are exergonic and occur spontaneously at room temperature.

Table 1. Calculated ΔG values (at 298.15 K and 1 atm) for the electron transfer reactions from degraded anions of compounds **1** and **2** to unreacted neutral compounds.

Reaction	ΔG (RROH) (kJ/mol)	
	X = 	X = 
	Compound 1	Compound 2
$SiPc(OH)X^{\bullet-} + SiPcX_2 \rightarrow SiPc(OH)X + SiPcX_2^{\bullet-}$	-2.6	-12.3
$SiPc(OH)_2^{\bullet-} + SiPcX_2 \rightarrow SiPc(OH)_2 + SiPcX_2^{\bullet-}$	-7.6	-15.7
$SiPc(OH)_2^{\bullet-} + SiPc(OH)X \rightarrow SiPc(OH)_2 + SiPc(OH)X^{\bullet-}$	-4.9	-3.4

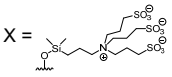
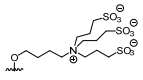
We next consider the reduction of $SiPc(OX)_2$ by e^-_{aq}. Because directly considering the energy of e^-_{aq} by quantum chemical calculations is difficult, the vertical and/or adiabatic electron affinities (VEA/AEA) are often compared with the vertical binding energy (VBE) of e^-_{aq}. The VEA and AEA of $SiPc(OX)_2$ are defined by

VEA = E(optimized $SiPc(OX)_2$) – E($SiPc(OX)_2^-$ at optimized $SiPc(OX)_2$ structure) (3)

AEA = E(optimized $SiPc(OX)_2$) – E(optimized $SiPc(OX)_2^-$) (4)

Table 2 summarizes the VEA (at internal energy) and AEA (at free energy at 298.15 K and 1 atm) of compounds **1** and **2** and their mono-degraded products. Because VEA and AEA are almost independent of the axial ligand, the reduction efficiency by e^-_{aq} is considered to be similar among the four axial ligands. The VBE of e^-_{aq} has been reported to be 3.3–3.9 eV, and the latest reported best estimate is 3.7 eV.[19] If the energy matches the VEA of a neutral solute, the electron attachment to the solute is enhanced because of the resonance phenomenon.[20] All of the VEA values are 3.6-3.7 eV, consistent with the experimentally reported VBE of an e^-_{aq}. This fact suggests that the reduction of $SiPc(OX)_2$ by e^-_{aq} is accelerated by the resonance effect. As for the reducing ability of the CO_2 anion radical, its VBE is 1.9 eV, which is smaller than the VEA of compounds **1** and **2**. Therefore, CO_2 anion radicals can also easily reduce compounds **1** and **2**.

Table 2. Calculated vertical electron affinity (VEA) of compounds **1** and **2**.

Reaction	VEA (eV)	
	X = 	X = 
	Compound 1	Compound 2
$SiPcX_2 \rightarrow SiPcX_2^{\bullet-}$	3.720	3.754
$SiPc(OH)X \rightarrow SiPc(OH)X^{\bullet-}$	3.664	3.672

From these results, as well as those in our previous report,[13] the putative mechanism of the radical chain reaction is shown in Fig. 6. $SiPc(OX)_2$ is effectively reduced by e^-_{aq}, which are

generated after X-ray irradiation of the water solvent in the resonant mechanism. The degradation of $[SiPc(OX)_2]^{\bullet-}$ to $[SiPc(OH)(OX)]^{\bullet-}$ and $[SiPc(OH)(OX)]^{\bullet-}$ to $[SiPc(OH)_2]^{\bullet-}$ proceeds through the same mechanism detailed in our previous report on IR700 degradation triggered by NIR irradiation. The generated $[SiPc(OH)(OX)]^{\bullet-}$ and $[SiPc(OH)_2]^{\bullet-}$ further reduces unreacted $SiPc(OH)(OX)$ or $SiPc(OX)_2$ if the association occurs within the lifetime of the anion radicals, leading to a radical chain reaction.

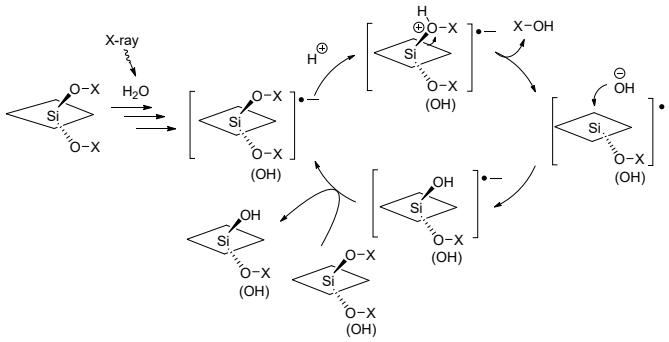


Fig. 6. Plausible chain reaction mechanism of compounds **1** and **2** upon X-ray irradiation. Compounds **1** and **2** receive an electron from a reducing species generated via water radiolysis to produce the anion radicals. The axial ligand exchange, including protonation, cleavage, and coordination of a hydroxy ion, occurs spontaneously at room temperature, as in the photochemical reaction. The resultant anion radicals with a hydroxy group can transfer their electron to other compounds **1** and **2** in the ground state to produce new anion radicals.

Discussion

Prodrugs (or caged compounds) are molecules that have lost their efficacy or activity because their active site has been masked with a protecting group. This protective group is designed to be removed under specific conditions or environments at the target site of the drugs and is expected to reduce the systemic side effects of the drugs by lowering their off-target efficacy.[21] On the one hand, although metabolic enzymes are often used as the target molecules, the target enzymes can be expressed outside the lesion, leading to side effects. On the other hand, there are cases where no appropriate target molecules exist in the lesion. In addition, the enzymatic activities and the expression level vary among drug recipients. Consequently, the efficacy of the prodrugs that target metabolic enzymes is both unpredictable and uncontrollable. Thus, prodrugs that can be activated by external energy are desirable; they enable quantitative and spatiotemporal control of the activation, because the position, timing and irradiation dose can be flexibly determined. In this context, extensive efforts have been devoted to developing prodrugs with protective groups that can be dissociated by light. The protective groups include nitrobenzyl, coumarin, BODIPY and cyanine structures, which can be activated by incident light in the UV-to-NIR region.[3,10] The mechanism of photodissociation is based on the photochemical reaction after the protective groups are excited by light. Because UV-to-NIR light is attenuated by tissue absorption and scattering, photoactivation in deep sites is challenging. However, X-rays have a very short wavelength (0.001-0.1 nm) and high energy. The energy of X-rays is much greater than the energies associated with excitation, and organic compounds cannot be excited by X-ray irradiation. Thus, although X-rays are not readily absorbed by tissues and shows high tissue permeability, photoactivatable caged compounds cannot be used as X-ray-activatable caged compounds that operate via the same activation mechanism.

The literature includes two recent reports on X-ray-induced caged compounds based on small molecules that do not contain heavy atoms.[22,23] In one report, 3,5-dihydroxybenzyl carbamate is used as a caging group that can efficiently react with OH radicals, followed by a 1,6-elimination reaction to release the drugs.[22] In another report, some azido compounds were converted into aniline compounds in response to X-ray irradiation and were subsequently used as an X-ray-activatable caging group, although the reaction mechanism is unclear.[23] Both 3,5-dihydroxybenzyl carbamate and azido compounds have been applied to the development of caged fluorophores and caged drugs (prodrugs) and have been used in both in vitro and in vivo experiments. These studies have shown that X-ray irradiation successfully activates the caged compounds. In such a case, the X-ray irradiation dose (efficiency of activation) is important because the side effects of X-rays are problematic for translational applications. For both X-ray-activatable caging groups, more than several tens of grays of radiation dose are required to activate 10 μM of caged compounds in the cuvette. In the in vitro and in vivo experiments, X-ray-activatable compounds functioned at 4-6 Gy of X-ray radiation; however, the activation efficiency should be improved. These low activation efficiencies arise from the mechanisms based on a stoichiometric reaction. To achieve high activation efficiency, a molecular design that can amplify the input is needed.

In this study, we found the axial ligand cleavage reaction of SiPcs that proceeds in the radical chain reaction is triggered by X-ray irradiation. To elucidate the mechanism of the X-ray-induced axial ligand cleavage, we conducted X-ray irradiation experiments, ESR measurements and analyses of degraded products using compound **2** in the presence of scavengers of OH radicals and e^-_{aq}. The results show that e^-_{aq} (or CO_2 anion radicals in the presence of an OH radical scavenger) generated by water radiolysis react with compound **2** to produce anion radicals; the axial ligand is then protonated, followed by dissociation of the axial ligand and coordination of a hydroxy ion derived from water, leading to the production of hydrophobic $SiPc(OH)_2$. We have already reported that, in the same compounds, cleavage of the axial ligands occurs by NIR irradiation in the presence of an electron donor.[16] A comparison of the two reactions reveals that the initial trigger differs, whereas the subsequent reaction is identical. A computational investigation showed that the resultant anion radical ($[SiPc(OX)(OH)]^{\bullet-}$ or $[SiPc(OH)_2]^{\bullet-}$) further reacts with SiPc in the ground state, leading to a radical chain reaction. The chain reaction of axial ligand cleavage can also be triggered by NIR light, but the reaction yields using a light dose were difficult to determine. Thus, the results suggested that the axial ligand

cleavage deep in the body can be triggered by X-ray irradiation instead of NIR irradiation, as we previously predicted.[13]

A stoichiometric analysis of the radical chain reaction using compound **2** was also performed. When a 500 μM solution of compound **2** was irradiated at 50 Gy under condition II, which corresponds to a total of 28 μM of reducing species according to the G-values, compound **2** completely disappeared. The results suggest that the radical chain reaction in compound **2** proceeds with a turnover rate greater than 17 times. In the case of 5 μM compound **2**, only 4 Gy of irradiation (corresponding to 2.2 μM of reducing species) is sufficient to release all of the axial ligands, indicating a turnover rate of approximately 2 times. The turnover rate in this case is lower than in the former case, suggesting that the concentration of compound affects the turnover rate of the chain reaction. Nevertheless, the activation efficiency is at least three times greater than those of previously reported X-ray-activatable compounds.[22,23] Accordingly, our compound that can be activated with a lower radiation dose exhibits excellent properties. Also, because we have elucidated the mechanism of the cleavage reaction, we can optimize the structure. For example, structures that can more easily receive electrons can be explored via computational investigations. The SiPcs could be used as an X-ray-activatable caged compound if a drug is attached at the axial position. The literature includes no reports on chain reactions triggered by reactions with e^-_{aq} or based on SiPc compounds. Therefore, our findings should pave the way to the development of X-ray-activatable systems based on small compounds.

Conclusions

Herein, we investigated X-ray-induced axial ligand cleavage of SiPc with siloxy, alkoxy, oxycarbonyl and phenoxy groups as axial ligands. Our results showed that the axial ligands of all compounds were cleaved by e^-_{aq} or CO_2 anion radicals. Quantitative analysis indicated that the reaction proceeds via a radical chain reaction. In particular, compound **2** demonstrated the highest efficiency, and the radical chain reaction in compound **2** proceeded with a turnover rate as high as 17 times. From the experimental and theoretical investigations, the following mechanism was suggested. Compound **2** receives e^-_{aq} to produce anion radicals, followed by protonation of the axial ligands. Then, axial ligand exchange, including cleavage of the axial ligand and coordination of OH^- ions, occurs and the resultant anion radicals donate electrons to compound **2** in the ground state, again producing the initial anion radical. Because axial ligand cleavage has been utilized in the molecular design of caged compounds, we expect that the radical chain reaction of SiPcs will be applicable to the development of X-ray-activatable functional molecules for clinical use in the future.

Author contributions

Conceptualization, H.T. and M.O.; methodology, H.T. and M.O.; investigation, H.T., S.M., O.I., M.K., K.S., M.Y., K.N., M.S., N.M. and T.T.; formal analysis, M.K., K.S. and T.T.; data curation, H.T., S.M., O.I., M.K., K.S. and M.Y.; writing–original draft preparation, H.T.; writing–review and editing, H.T., K.N. and M.O.; visualization, H.T. and S.M.; supervision, M.O.; project administration, M.O.; funding acquisition, H.T. and M.O. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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