Theoretical and Experimental Studies on the Near-Infrared Photoreaction Mechanism of a Silicon Phthalocyanine Photoimmunotherapy Dye: Photoinduced Hydrolysis by Radical Anion Generation

Masato Kobayashi,*[a, b] Mei Harada,[c] Hideo Takakura,[c] Kanta Ando,[c] Yuto Goto,[c] Takao Tsuneda,[a, d] Mikako Ogawa,*[c] and Tetsuya Taketsugu[a, b]

Ligand release from IR700, a silicon phthalocyanine dye used in near-infrared (NIR) photoimmunotherapy, initiates cancer cell death after NIR absorption, although its photochemical mechanism has remained unclear. This theoretical study reveals that the direct Si-ligand dissociation by NIR light is difficult to activate because of the high dissociation energy even in excited states, i.e., >1.30 eV. Instead, irradiation generates the IR700 radical anion, leading to acid-base reactions with nearby water molecules (i.e., calculated pK_b for the radical anion is 7.7) to produce hydrophobic ligand-released dyes. This suggests two possibilities: (1) water molecules participate in ligand release and (2) light is not required for Si–ligand dissociation as formation of the IR700 radical anion is sufficient. Experimental evidence confirmed possibility (1) by using ^{18}O-labeled water as the solvent, while (2) is supported by the pH dependence of ligand exchange, providing a complete description of the Si-ligand bond dissociation mechanism.

Near-infrared photoimmunotherapy (NIR-PIT) is a novel and cancer-specific therapy that uses monoclonal antibody conjugates of IRDye 700DX (IR700), a silicon phthalocyanine (SiPc) dye (**1**; Scheme 1) that absorbs NIR light.[1–5] Its cytotoxic effects are induced only when the conjugates are bound to the antigen on the target cancer cell membrane and irradiated with NIR light.[6,7] In NIR-PIT, the cell membrane is believed to be physically damaged upon irradiation with NIR light. After the irradiation, water flows into the cell, thus ions and small molecules penetrate the cell membrane, leading to necrotic/immunogenic cell death. A recent study demonstrated that photodamage of the cell membrane is attributed to Si–O bond dissociation from Si located at the center of the axial ligand in IR700 in the presence of electron donors, which is then followed by aggregation of the conjugates.[8] This Si–O dissociation reaction has been explained as proceeding from NIR-induced photodissociation. However, the dissociation reaction mechanism after light irradiation has never been clarified. Note that the NIR-induced aggregation of antibodies released from the IR700 dye, which is derived from the reaction yielding hydrophobic product (**4**; Scheme 2), has been experimentally observed to occur only in the presence of electron donors.[8] This experimental study also demonstrated that the use of a deoxygenated solution enhances the reaction rate, which is indicative of the formation of radical anion intermediates.[8,9]

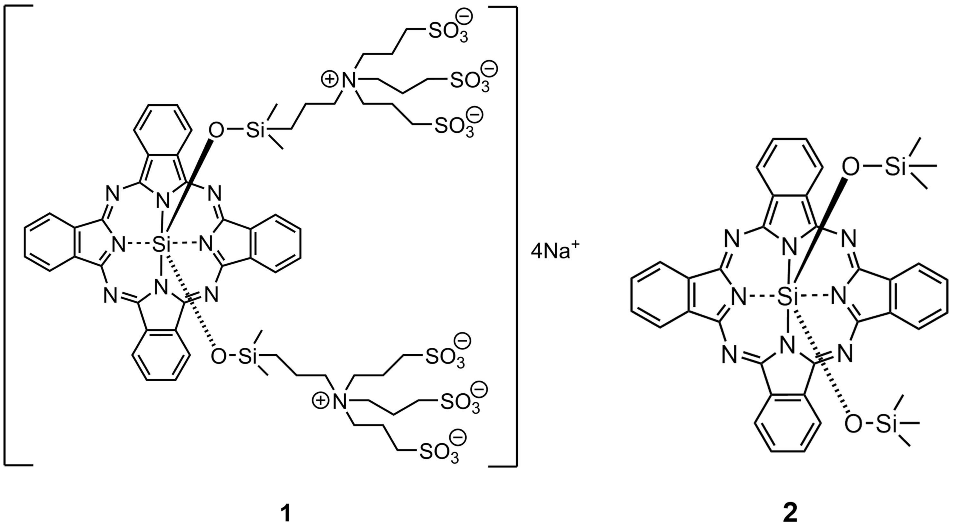
In this study, we theoretically and experimentally investigate the Si–O dissociation reaction mechanism after NIR irradiation of IR700. We explore the contributions of photoexcitation, solvent water molecules, and electron donors to Si–O dissociation.

Vertical excitation energy calculations were first performed for the IR700 dye model without a conjugate linker on the phthalocyanine ring (**1**) and its core model (**2**; Scheme 1). The calculated results show that truncation of the molecule barely affects the vertical excitation energies (see Section S-II in the Supporting Information). We therefore adopted the truncated core model **2** for the calculations. The calculated Si–O dissociation curves also showed that participation of a nearby water molecule significantly lowers the activation energy for Si–O dissociation (see Section S-III in the Supporting Information). Based on these theoretical calculations, we experimentally examined the participation of nearby water molecules by following the reaction in Scheme 2 using ^{18}O-labeled water ([^{18}O]H_2O) as the solvent. Figure 1 shows the MALDI-TOF mass spectra of compound **1** irradiated in a sodium phosphate buffer solution prepared with [^{18}O]H_2O or H_2O in the presence of cysteine under hypoxic conditions. NIR photoirradiation of compound **1** in [^{18}O]H_2O gives the parent and fragment peaks

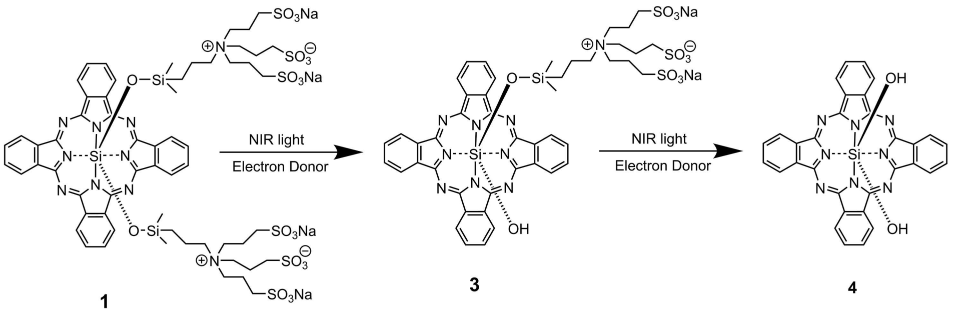
---

[a] Dr. M. Kobayashi, Prof. T. Tsuneda, Prof. T. Taketsugu
Faculty of Science
Hokkaido University
Sapporo 060-0810 (Japan)
E-mail: k-masato@sci.hokudai.ac.jp
[b] Dr. M. Kobayashi, Prof. T. Taketsugu
WPI-ICReDD
Hokkaido University
Sapporo 001-0021 (Japan)
[c] M. Harada, Dr. H. Takakura, K. Ando, Y. Goto, Prof. M. Ogawa
Faculty of Pharmaceutical Sciences
Hokkaido University
Sapporo 060-0812 (Japan)
E-mail: mogawa@pharm.hokudai.ac.jp
[d] Prof. T. Tsuneda
Graduate School of Science Technology, and Innovation
Kobe University
Kobe 657-8501 (Japan)

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cplu.202000338



Scheme 1. IR700 dye without a conjugate linker on the phthalocyanine ring (1) and its core model (2) used for the computational investigation.



Scheme 2. Photodegradation scheme of IR700 dye without a conjugate linker (1) to mono-substituted hydroxy (3) and bis-substituted hydroxy (4) dyes.

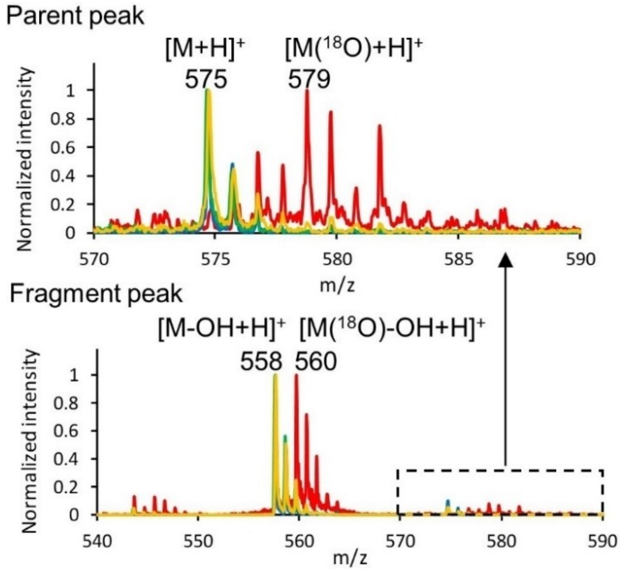
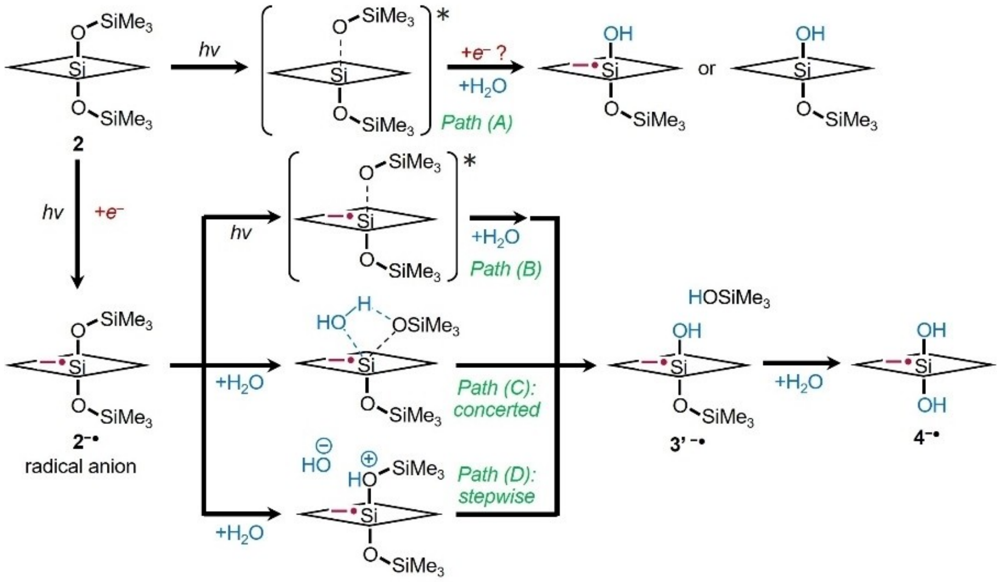


Figure 1. MALDI-TOF mass spectra of compound 4. Photocleaved product **4** obtained from compound **1** after NIR irradiation in [^{18}O]H_2O (red) and H_2O (blue). Authentic compound **4** in H_2O (green) and after heating at 100 °C in [^{18}O]H_2O for 1 h (yellow).

of photocleaved product **4** containing ^{18}O atoms. Note that the peaks of product **4** in H_2O are consistent with those of authentic compound **4**. On the other hand, ^{18}O-labeled peaks were not detected for authentic compound **4** heated in [^{18}O]H_2O. This suggests that no ligand exchange occurs for compound **4** in water. These results clearly show that the OH ligands are derived from the reaction in Scheme 2 with water as the solvent, while also indicating that the reaction proceeds through bond dissociation of the central Si of the phthalocyanine and the apical silyloxy ligand O. The mediation of Si–O dissociation with a water molecule was also suggested by Anderson *et al.* for a SiPc compound with alkoxy and phenoxy ligands,[10] further supporting the current evidence that Si–O dissociation occurs through NIR irradiation-supported hydrolysis.

Scheme 3 summarizes plausible reaction mechanisms based on the above-mentioned theoretical and experimental evidence. Two significant points are revealed: (i) the role of NIR light and (ii) the contribution of a water molecule to the reaction. Regarding point (i), it is presumed that NIR light contributes only to production of radical anion species $2^{-\bullet}$ [paths (C) and (D)], whereby radical anion formation is observed after NIR irradiation in the experimental absorption spectrum.[8] However, it may support direct Si–O photodissociation through



Scheme 3. Plausible reaction mechanisms of IR700 model 2 to yield ligand-substituted compound 4.

hydrolysis [paths (A) and (B)] as well. As for point (ii), a water molecule can contribute to the reaction in at least two plausible mechanisms: concerted [path (C)] and stepwise [path (D)].

First, we explored the mechanisms of paths (A) and (B), i.e., photodissociation of the Si–O bond of neutral compound **2** and its anion radical **2**$^{-\bullet}$. It has been proposed that photochemical Si–C bond cleavage proceeds by a dissociation channel through a charge-transfer excited state for a related SiPc dye with an alkyl ligand.[11,12] Figure 2(A) shows the ground state and excited state potential energy curves of neutral compound **2** calculated with long-range correction (LC)[13] for density functional theory (DFT) and time-dependent DFT (TDDFT), which are established to potentiate quantitative analyses of charge-transfer processes (see Section S–I in the Supporting Information for computational details). Since we allowed excitations from spin-unrestricted ground states in the LC-TDDFT calculations, we analyzed the expected value of the total spin squared operator (S^2) for each state, represented by color depth (grayscale) in Figure 2; that is, the excitation plots that are light gray in color contain large spin contamination and should not be interpreted as pure singlet excitations. The Franck-Condon excitation energy to the lowest singlet excited state is 2.06 eV (601 nm), which is slightly higher than that of the experimental NIR absorption (~680 nm, ~1.82 eV). Note that the first excitation energies of compounds **1** and **2** by the spin-restricted LC-TDDFT method, given in Table S1 in the Supporting Information, are 1.86 and 1.85 eV, respectively. This result therefore agrees with the experimental value, which supports the effectiveness of the adopted functional and implies that the slight discrepancy of the excitation energy in Figure 2 is due to spin contamination. The potential energy curves of the excited states are nearly parallel to that of the ground state. We found only π-π^* excitations of the phthalocyanine with no charge-transfer excitation in low-lying excited states. Similar excitations occur for anion radical form **2**$^{-\bullet}$, as depicted in Figure 2(B), though the dissociation energies are lower; the lowest Franck–Condon singlet excitation energy is 1.30 eV. This indicates that the photodissociation mechanisms shown in paths (A) and (B) of Scheme 3 for Si–O cleavage are more difficult to attain as opposed to the proposed mechanism for Si–C cleavage. We address the reason for the difference between Si–O and Si–C cleavage from the viewpoints of molecular orbitals and electron density in Section S-IV in the Supporting Information.

Next, we examined the concerted ligand substitution reaction mechanism with a water molecule in path (C) of Scheme 3. The transition states of the concerted reaction were determined for neutral compound **2** and its radical anion **2**$^{-\bullet}$. The calculated results show that the reaction is slightly endergonic (+0.54 and +0.76 kJ·mol^{-1} for **2** and **2**$^{-\bullet}$, respectively), while exhibiting very high Gibbs activation energies (209.0 and 161.8 kJ·mol^{-1}, respectively), which are too high to proceed at room temperature. Note that the reported activation energies of concerted ligand substitution reactions of SiPc with phenoxy ligand are 147.3 and 121.3 kJ·mol^{-1} for neutral and radical anion species, respectively,[10] which are much lower than those of **2** and **2**$^{-\bullet}$, thus indicating that the concerted reaction of path (C) barely proceeds.

Instead of path (C), we chose to investigate the stepwise reaction mechanism of path (D) in Scheme 3; the hypothetical mechanism for the stepwise reaction of the radical anion form (**2**$^{-\bullet}$) is shown in Scheme 4. In this scenario, the zwitterionic oxonium intermediate (**5**$^{\bullet}$) is produced from the acid-base equilibrium of **2**$^{-\bullet}$ in water, and then homolytic bond cleavage of the apical Si–O bond produces the neutral SiPc radical (**6**$^{\bullet}$) and $SiMe_3OH$. Finally, the hydroxide ion (OH^-), which is generated from water during the prior acid-base reaction, attacks the open ligand site of the SiPc radical (**6**$^{\bullet}$) to produce the radical anion form of the mono-substituted product (**3'**$^{-\bullet}$).

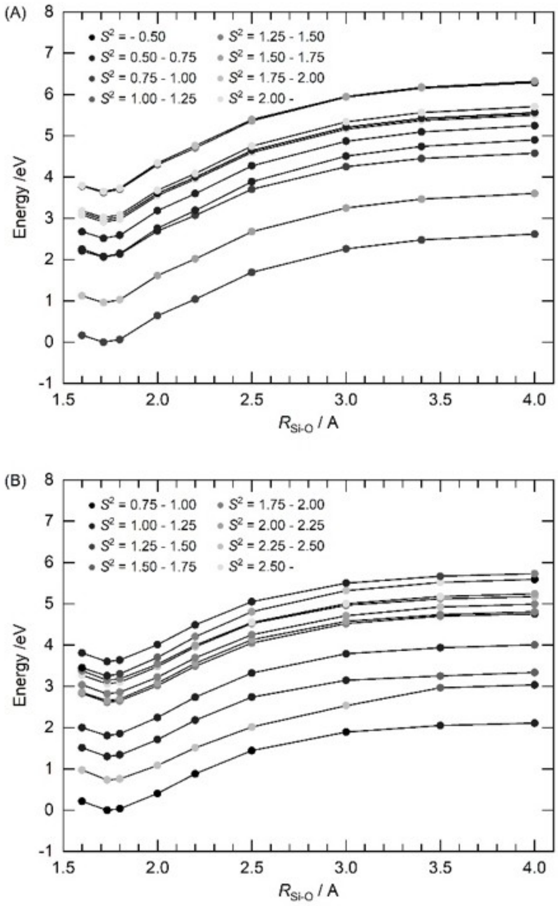
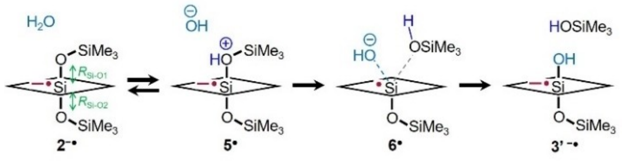


Figure 2. Ground state and low-lying excited state potential energy curves for Si–O dissociation of (A) compound **2** and (B) its radical anion $\mathbf{2}^{-\bullet}$. The geometrical parameters other than a Si–O bond length are relaxed in the ground state. The spin-unrestricted LC-BLYP/(aug-)cc-pVDZ level of computation was adopted for obtaining ground-state relaxed potential energy curves and the excited state energies were obtained with the TDDFT calculation with the same functional and basis set for the fixed geometries. The water solvent effect was incorporated using IEFPCM method. Here, aug-cc-pVDZ basis set was adopted for N and O atoms, while the cc-pVDZ basis set was utilized for all other atoms. The color depth (grayscale) indicates the expected S^2 value, i.e., the magnitude of spin contamination is higher for light gray and lower for black. The minimum energy geometry at the S_1 state is also discussed in Section S-VII in Supporting Information.



Scheme 4. Stepwise mechanism of the reaction of radical anion complex $2^{-\bullet}$ with a water molecule.

Figure 3 illustrates the Gibbs energy diagram for the reaction of SiPc ($\mathbf{2}/\mathbf{2}^{-\bullet}$) with a nearby water molecule. To examine the large explicit solvation effect of water as a solvent (see Section S–V in the Supporting Information), the following reaction formula was examined

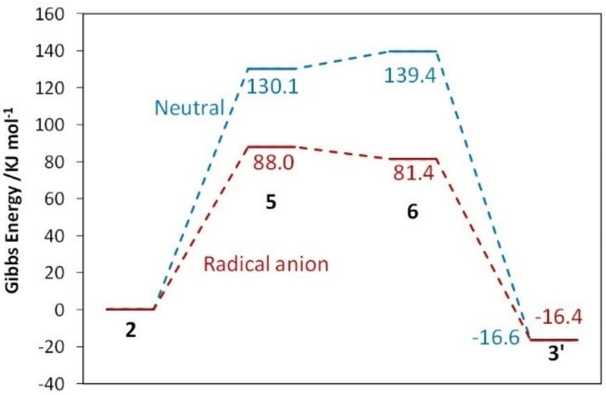


Figure 3. Potential energy diagram for the reaction of SiPc radical anion ($2^{-\bullet}$) and a water molecule. The spin-unrestricted LC-BLYP/(aug-)cc-pVDZ level of computation was adopted with IEFPCM water solvent effect for obtaining optimized geometries. We here only discuss the equilibrium between **2** and **5**, because it is a typical acid/base reaction in diluted water solution.

$$\begin{aligned}&\mathbf{2}^{-\bullet}{}_{(aq)} + (H_2O)_{3(aq)} \rightleftharpoons \mathbf{5}^{\bullet}{}_{(aq)} + (H_2O)_2OH^-{}_{(aq)}\\ &\rightarrow \mathbf{6}^{\bullet}{}_{(aq)} + (H_2O)_{2(aq)} \rightarrow \mathbf{3}'^{-\bullet}{}_{(aq)} + SiMe_3OH + (H_2O)_{2(aq)}\end{aligned} \qquad (1)$$

The zwitterionic oxonium intermediate ($\mathbf{5}^{\bullet}$) with OH^- is $88.0\ kJ\cdot mol^{-1}$ higher in energy than the reactant state ($\mathbf{2}^{-\bullet}+H_2O$), which is significantly lower than that of neutral SiPc (**2**; $130.1\ kJ\cdot mol^{-1}$). The corresponding pK_b values are 7.7 and $11.4\ mol\cdot L^{-1}$ for $\mathbf{2}^{-\bullet}$ and **2**, respectively. These results imply production of the oxonium intermediate ($\mathbf{5}^{\bullet}$). While Si–O dissociated structure ($\mathbf{6}^{\bullet}$) lies at slightly lower Gibbs energy than the oxonium intermediate ($\mathbf{5}^{\bullet}$), the corresponding compound for the neutral reaction ($\mathbf{6}^{+}$) has slightly higher Gibbs energy than the oxonium intermediate ($\mathbf{5}^{+}$). Addition of OH^- to neutral SiPc radical ($\mathbf{6}^{\bullet}$) is highly exergonic ($-97.7\ kJ\cdot mol^{-1}$), producing mono-substituted $\mathbf{3}'^{-\bullet}$, though as a whole; the overall reaction is slightly exergonic ($-16.4\ kJ\cdot mol^{-1}$). The calculated small energy barrier for the entire process of this reaction indicates that ligand exchange proceeds after NIR-induced SiPc radical anion ($\mathbf{2}^{-\bullet}$) formation at room temperature (or *in vivo)*. The Si–O bond length variations along the reaction path of Scheme 4 are discussed in Section S-VI in the Supporting Information. In addition, the acid/base reaction energy at the S_1 state ([2]* to [5]*) is investigated in Section S-VII in the Supporting Information. Further reaction yielding bis-substituted **4** from $\mathbf{3}'^{-\bullet}$ proceeds through the same reaction mechanism (see Section S-VIII in the Supporting Information).

The above mechanism suggests that as the pH increases, the equilibrium between $\mathbf{2}^{-\bullet}$ and $\mathbf{5}^{\bullet}$ tends to favor $\mathbf{2}^{-\bullet}$, resulting in slower ligand exchange. This reaction was further evaluated at different pH by monitoring the change in absorbance of IR700, as depicted in Figure 4. The photoproduct **4** does not possess the hydrophilic axial ligands and is insoluble in aqueous solution due to hydrophobicity of the phthalocyanine ring. Therefore, when the photocleavage reaction proceeds, the absorbance peak of IR700 at 680 nm, which corresponds to the π-π* excitations of the phthalocyanine ring, decreases since the photoproduct **4** precipitates out of solution. As a result, the

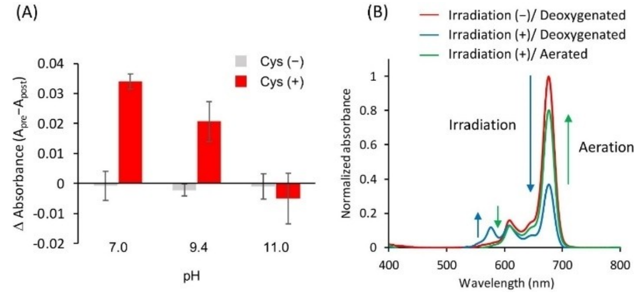


Figure 4. (A) Change in absorbance is determined by the absorbance of 1 μM IR700 at 680 nm before NIR irradiation with absorbance after NIR irradiation (690 nm, 20 mWcm^{-2}, 10 min) and aeration with or without 1 mM cysteine. (B) Absorption spectra of 1 μM IR700 with 1 mM cysteine at pH 7.0. The deoxygenated solution before NIR irradiation (red), the deoxygenated solution after NIR irradiation (690 nm, 20 mWcm^{-2}, 10 min) (blue), and the aerated solution after NIR irradiation (green).

electron donor-dependent absorbance decreases after NIR irradiation and aeration, whereas the absorbance barely changes at pH 11, even in the presence of an electron donor. The absorption spectra also show that the absorbance peak at 578 nm, which is very sensitive to oxygen, appears after NIR irradiation. This indicates that photocleavage proceeds after generation of radical anion species. These results demonstrate that protonation of oxygen in the Si–O bond is required for photocleavage via the radical anion, which is further accelerated at lower pH. The change of the absorption spectra of IR700 at various pH values, including weakly acidic condition (pH 6.0), is shown in Section S-IX in the Supporting Information.

We have theoretically and experimentally investigated the Si–O bond dissociation mechanism of the silicon phthalocyanine (SiPc) dye IR700, which is the key molecule of highly-selective cancer phototherapy, under near-infrared (NIR) light irradiation. As a result, we have revealed that Si–O bond dissociation does not proceed by direct NIR photodissociation, but rather through the following processes. After NIR light-induced excitation of the IR700 molecule, an electron is donated to IR700 from a reducing agent to yield the radical anion form of IR700. Then, a silyloxy ligand of the IR700 radical anion is protonated by an acid-base reaction with water. This protonation induces the dissociation of a central Si–O bond. The open ligand site after Si–O bond dissociation then becomes occupied by an OH$^-$ generated by the prior auto-ionization of a water molecule, which forms the mono-substituted intermediate **3**. The other silyloxy ligand then undergoes the same bond dissociation process to yield the hydrophobic SiPc **4**. This mechanism is experimentally supported by the reaction product identified using ^{18}O-labeled water as the solvent and by measuring the pH dependence of the product yield. We expect that with some modifications, IR700 cancer therapy can be activated even without NIR light based on this mechanism, and therefore, that this therapy could be efficacious even in deep tissues, where NIR light cannot readily penetrate, although this assumption could not be fully proven by the present study. Simple changes that are feasible to investigate are the use of a stronger electron donor to directly reduce IR700 without NIR irradiation and synthetic modification of IR700 to obtain a lower LUMO energy in order to facilitate reduction with a moderate electron donor.

Acknowledgments

We thank Rakuten Medical Japan for financial support. Some calculations were performed using the computer facilities at Research Center for Computational Science, Okazaki and at Research Institute for Information Technology, Kyushu University, Japan. This work was supported in part by JST-PRESTO (JPMJPR15P5), JST-CREST (JPMJCR1902), JSPS KAKENHI (17H01188), and the Photo-excitonix Project in Hokkaido University. The Institute for Chemical Reaction Design and Discovery (ICReDD) was established by the World Premier International Research Initiative (WPI), MEXT, Japan.

Conflict of Interest

The authors declare no conflict of interest.

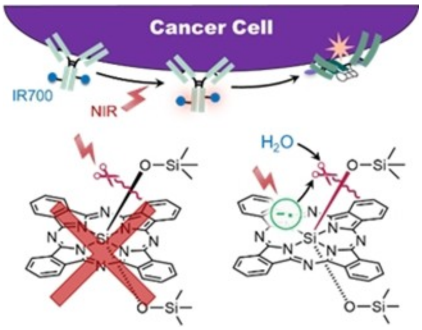
Keywords: bond dissociation · cancer photoimmunotherapy · density functional calculation · near-infrared dyes · radical anions · silicon phthalocyanine

[1] M. Mitsunaga, M. Ogawa, N. Kosaka, L. T. Rosenblum, P. L. Choyke, H. Kobayashi, *Nat. Med.* **2011**, *17*, 1685.
[2] K. Sato, N. Sato, B. Xu, Y. Nakamura, T. Nagaya, P. L. Choyke, Y. Hasegawa, H. Kobayashi, *Sci. Transl. Med.* **2016**, *8*, 352ra110.
[3] N. Shirasu, H. Yamada, H. Shibaguchi, M. Kuroki, M. Kuroki, *Int. J. Cancer* **2014**, *135*, 2697.
[4] H. Jing, C. Weidensteiner, W. Reichardt, S. Gaedicke, X. Zhu, A.-L. Grosu, H. Kobayashi, G. Niedermann, *Theranostics* **2016**, *6*, 862.
[5] T. Nagaya, Y. Nakamura, K. Sato, T. Harada, P. L. Choyke, J. W. Hodge, J. Schlom, H. Kobayashi, *Oncotarget*, **2017**, *8*, 8807.
[6] K. Nakajima, H. Takakura, Y. Shimizu, M. Ogawa, *Cancer Sci.* **2018**, *109*, 2889.
[7] M. Ogawa, Y. Tomita, Y. Nakamura, M.-J. Lee, S. Lee, S. Tomita, T. Nagaya, K. Sato, T. Yamauchi, H. Iwai, A. Kumar, T. Haystead, H. Shroff, P. L. Choyke, J. B. Trepel, H. Kobayashi, *Oncotarget* **2017**, *8*, 10425.
[8] K. Sato, K. Ando, S. Okuyama, S. Moriguchi, T. Ogura, S. Totoki, H. Hanaoka, T. Nagaya, R. Kokawa, H. Takakura, M. Nishimura, Y. Hasegawa, P. L. Choyke, M. Ogawa, H. Kobayashi, *ACS Cent. Sci.* **2018**, *4*, 1559.
[9] E. D. Anderson, A. P. Gorka, M. J. Schnermann, *Nat. Commun.* **2016**, *7*, 13378.
[10] E. D. Anderson, S. Sova, J. Ivanic, L. Kelly, M. J. Schnermann, *Phys. Chem. Chem. Phys.* **2018**, *20*, 19030.
[11] T. Doane, Y. Cheng, N. Sodhi, C. Burda, *J. Phys. Chem. A* **2014**, *118*, 10587.
[12] B. Maiti, A. K. Manna, C. McCleese, T. L. Doane, S. Chakrapani, C. Burda, B. D. Dunietz, *J. Phys. Chem. A* **2016**, *120*, 7634.
[13] T. Tsuneda, K. Hirao, *WIREs Comput. Mol. Sci.* **2014**, *4*, 375.

Manuscript received: April 28, 2020
Revised manuscript received: May 1, 2020

COMMUNICATIONS

The computational and experimental studies demonstrated that the mechanism of action for near-infrared (NIR) photoimmunotherapy using the key Si phthalocyanine dye, IR700, is a result of the formation of IR700 radical anion. The dissociation of the axial ligands from IR700 has been established as the initiation of cancer cell death, though understanding the mechanism to bring this about has remained elusive. The ligand dissociation proceeds by the hydrolysis of this radical anion, rather than any dissociative excited-state channel.



Dr. M. Kobayashi, M. Harada, Dr. H. Takakura, K. Ando, Y. Goto, Prof. T. Tsuneda, Prof. M. Ogawa*, Prof. T. Taketsugu*

1 – 6

Theoretical and Experimental Studies on the Near-Infrared Photoreaction Mechanism of a Silicon Phthalocyanine Photoimmunotherapy Dye: Photoinduced Hydrolysis by Radical Anion Generation