

Confocal Laser Scanning Microscope Analysis of Antimony Porphyrin Chromophore Immobilized on Silica-gel Beads

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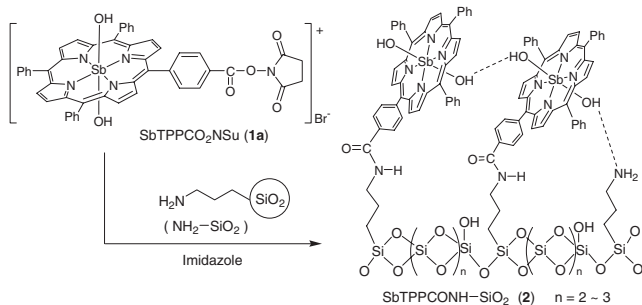
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Dihydroxoantimony(V) tetraphenylporphyrin chromophore (SbTPP) was immobilized on 3-aminopropyl-silica gel ($\text{NH}_2\text{-SiO}_2$) by the reaction of dihydroxoantimony [4-(*N*-succinimidyloxycarbonyl)phenyl]triphenylporphyrin with $\text{NH}_2\text{-SiO}_2$. Spectroscopic analysis of the complex by confocal laser scanning microscope suggested the interaction of SbTPP with the residual amino group on SiO_2 . The photoreaction of the complex with Et_2NH induced the demetallation.

Metalloporphyrin chromophores play important roles as energy-harvest pigments in natural photosynthesis¹ and the photosensitizer operating under visible light irradiation.^{2,3} It is well known that dihydroxoantimony tetraphenylporphyrin halide (SbTPP) has strong oxidation ability among the metalloporphyrin complexes prepared so far. On the other hand, silica-gel (SiO_2) has high transparency of visible light and binding ability of the cationic chromophore by a Coulombic force and hydrogen bonding. Therefore, we have prepared the photocatalysts by the fixation of SbTPP on SiO_2 to apply to the dechlorination,² the epoxidation,³ and sterilization.⁴ However, the binding force of SbTPP chromophore to SiO_2 was not so strong, since a Coulombic force and hydrogen bonding were weak. So, we here immobilized SbTPP chromophore on SiO_2 beads through a covalent bond.⁵



Scheme 1. Synthetic route to SbTPPCONH- SiO_2 (**2**).

Micrometer size of 3-aminopropyl-silica gel ($\text{NH}_2\text{-SiO}_2$, diameter = $56.7\ \mu\text{m}$)⁶ with a sharp size distribution was selected as a support of a chromophore. As the chromophore being capable of linking with $\text{NH}_2\text{-SiO}_2$, we used an active ester, SbTPPCO₂NSu (**1a**) (Scheme 1). The **1a** was prepared by the reaction of *N*-hydroxysuccinimide with dihydroxoantimony(V) (4-carboxyphenyl)triphenylporphyrin, which was prepared by the reaction of SbBr_3 with *meso*-(4-carboxyphenyl)triphenylporphyrin followed by the treatment with Br_2 and the subsequent hydrolysis. The $\text{NH}_2\text{-SiO}_2$ was reacted with a given molar

equivalent (a ; 2–20 meq) of **1a** to the amino group of the $\text{NH}_2\text{-SiO}_2$ in the presence of imidazole to give the immobilized complex (SbTPPCONH- SiO_2 ; **2**). The bond formation was confirmed by FT-IR spectra that showed the appearance of amide bond at 1630 and $1580\ \text{cm}^{-1}$.

Absorption spectra of a bead of **2** were measured by microscopic analysis on confocal laser scanning microscope (CLSM; Olympus FV-300) equipped with a multi-spectrophotometer (Seki Technotron, STFL-250). The Q band of porphyrins appeared at 550 and $590\ \text{nm}$, which were very similar to that of dihydroxoantimony [4-(*n*-propylcarbamoyl)phenyl]triphenylporphyrin (SbTPPCONHPr; **1b**) in solution (552 and $595\ \text{nm}$, respectively). The diameters ($b\ \text{cm}$) of a bead of **2** were measured by CLSM. The values (A/b), which were obtained by the division of the observed absorbances (A) at $552\ \text{nm}$ by b , were plotted against the value a , giving a good linear correlation until a value reached to $12\ \text{meq}$, as shown in Figure 1. From the slope ($5.52 \times 10^3\ \text{eq}^{-1}\cdot\text{cm}^{-1}$) of the plots, the immobilization yield (γ) of **1a** on $\text{NH}_2\text{-SiO}_2$ was estimated to be 23.8% .⁷ Thus, the loading amount ($x\ \text{mol/g}$) of SbTPP chromophore in $1\ \text{g}$ of SiO_2 can be calculated using $x = 2.53 \times 10^{-3}\gamma \cdot a$.

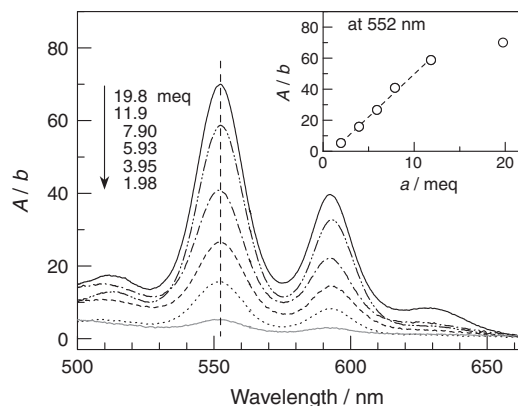


Figure 1. Absorption spectra of a bead of **2** in various a values. Inset shows the plots of A/b vs the a value.

The fluorescence spectra of a bead of **2** were remarkably different from that of **1b** in solution; new broad emission appeared near $630\ \text{nm}$ (Figure 2a). It is deduced that the SbTPP chromophores in the excited state interacted with other chromophores. When the residual amino group on a bead of **2** was protected by an acetylation with Ac_2O , the new emission at $630\ \text{nm}$ disappeared (Figure 2b). Therefore, the SbTPP chromophore interacted with the residual amino moieties through hydrogen bonds. Irrespective of the acetylation, however, the spectral shape was changed when more than $19.8\ \text{meq}$ of SbTPP chromophore

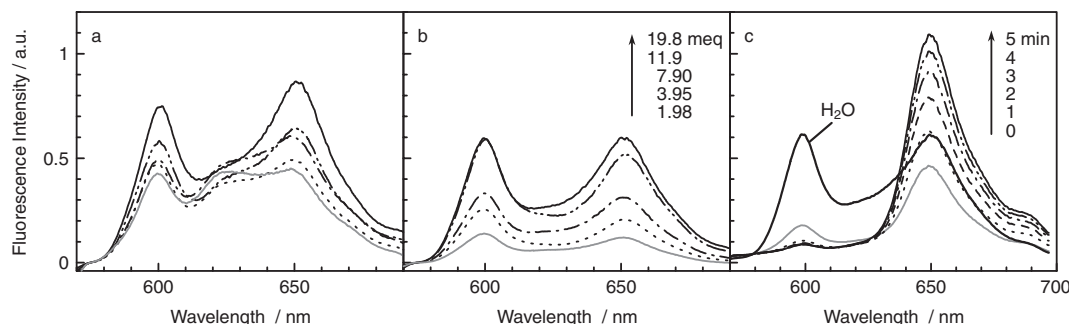


Figure 2. Fluorescence spectra of a bead of **2** (a) before and (b) after acetylation. (c) Fluorescence spectral change of a bead of the acetylated **2** in MeCN solution of 5 mmol/dm³ Et₂NH under irradiation of a laser light at 543 nm.

was loaded. In this case, it is attributable to the interaction between SbTPP chromophores.

The distance between aminopropyl groups was estimated as follows. When 2.53 mmol/g of the amino groups were contained on the NH₂-SiO₂, the composition ratio, SiO₂:HO-Si-C₃H₆NH₂, is calculated to be 6.9:1. If the SiO₂ moieties were arranged in two dimensions, the aminopropyl groups presumed to be apart from 2–3 units of SiO₂ each other. Therefore, the SbTPP chromophores located at the distance enough to interact with the residual amino moiety (Scheme 1).

For the photoreaction of microbeads, we used a microchannel reactor (MCR)^{8,9} that consisted of a narrow channel (width 190 μm, depth 85 μm, length 6 cm) and a neck (width and depth 20 μm) located at the middle point of the channel. An MeCN solution of Et₂NH (5 mmol/dm³) was introduced with micro-syringe pump into the MCR, where beads of the acetylated **2** ($x = 7.17 \mu\text{mol/g}$) was packed (Figure 3). He-Ne laser irradiation at 543 nm was performed on CLSM at the area of 30 μm squares, which was the comparable size of the beads. As increase of the irradiation time, the emissions from the surface of the catalyst at 600 nm decreased and new emission at 630 nm increased (Figure 2c). However, no spectral change was observed in non-irradiated beads. New emission can be assigned to be metal-free porphyrin chromophore (H₂TPPCONH-SiO₂) by the comparison of the absorption and fluorescence spectra with authentic sample. The immobilization through covalent bond makes the metal exchange of the SbTPP on beads possible.

Free energy change for the electron transfer from Et₂NH to the excited singlet state of a bead of **2** was calculated to be negative (−0.34 eV) by a Rehm–Weller equation using the oxidation potential of Et₂NH ($E_{1/2 \text{ ox}} = 1.01 \text{ V vs Ag/Ag}^+$)¹⁰ and the reduction potentials and the excitation energy of SbTPP chromophore.¹¹ Therefore, the photoinduced electron transfer from Et₂NH to SbTPP chromophore caused the reduction from Sb^V to Sb^{IV} to induce the demetallation from SbTPP chromophore. Thus, a combination of CLSM with MCR will be power-

ful tool for the analysis and photochemical reaction in micro-region on beads.

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References and Notes

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- 6 Aminopropyl silica gel (NH₂-SiO₂): Fuji Silysia, Average diameter (μm): 56.7, Area (m²/g): 272, Density (g/mL): 0.62, Content of NH₂ (mmol/g): 2.53.
- 7 According to Lambert–Beer's law (Eq 1) where A and b are absorbance and light path-length in cm, respectively, the A/b values are equal to the immobilized SbTPP concentration (c) that was related to the mol number (x) of SbTPP chromophore in 1 g of SiO₂. If the mol absorptivity (ϵ) of a bead of **2** is equal to that of **1b** ($1.48 \times 10^4 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$), the value of A/b can be related to equivalent (a) to the aminopropyl moiety by Eq 2 using the immobilization yield (γ) of **1a** on NH₂-SiO₂. Therefore, the slope of Figure 1 can be represented by Eq 3, resulting that γ is 23.8%. $A = \epsilon bc$ (Eq 1), $A/b = \epsilon c = 1.57\epsilon\gamma a$ (Eq 2) where $c = 1000Wx/V = 2.53W\gamma a/V = 1.57\gamma a$, $x = 2.53 \times 10^{-3}\gamma a$, and $W = 0.62 \text{ V}$. Slope = $1.57\epsilon\gamma$ (Eq 3), and $\gamma = \text{slope}/(1.57\epsilon)$.
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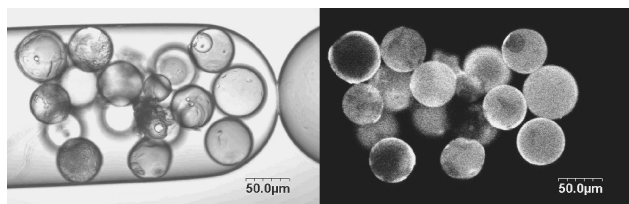


Figure 3. Microscopic image of the acetylated **2** in a MCR (left) and its CLSM fluorescence image (right).