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

Jin Matsumoto,* Tomokazu Fuchikawa, Yasuhiro Komiya, Yoshiyuki Fueda,† Tomoko Matsumoto, Tsutomu Shiragami, and Masahide Yasuda

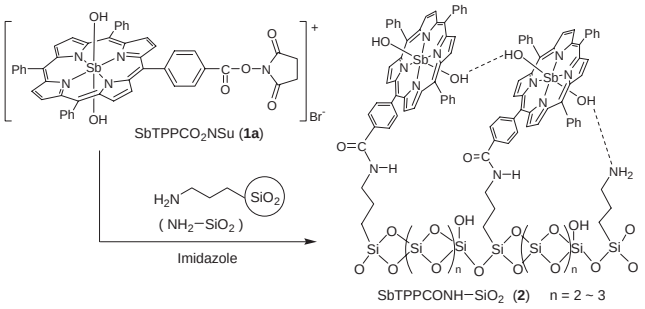
Department of Applied Chemistry, Faculty of Engineering, University of Miyazaki, Gakuen-Kibanadai, Miyazaki 889-2192

†*Fuji Silysia Chemical Ltd., 16303-3, Kihara, Hichiya, Hyuga, Miyazaki 883-0062*

(Received July 25, 2005; CL-050957)

Dihydroxoantimony(V) tetraphenylporphyrin chromophore (SbTPP) was immobilized on 3-aminopropyl-silica gel (NH_2–SiO_2) by the reaction of dihydroxoantimony [4-(*N*-succinimidyloxycarbonyl)phenyl]triphenylporphyrin with NH_2–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 photosynthesis[1] 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 (NH_2–SiO_2, diameter = 56.7 μm)[6] with a sharp size distribution was selected as a support of a chromophore. As the chromophore being capable of linking with NH_2–SiO_2, we used an active ester, $SbTPPCO_2NSu$ (**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 NH_2–SiO_2 was reacted with a given molar equivalent (*a*; 2–20 meq) of **1a** to the amino group of the NH_2–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 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 nm, which were very similar to that of dihydroxoantimony [4-(*n*-propylcarbamoyl)phenyl]triphenylporphyrin (SbTPPCONHPr; **1b**) in solution (552 and 595 nm, respectively). The diameters (*b* 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 nm by *b*, were plotted against the value *a*, giving a good linear correlation until *a* value reached to 12 meq, as shown in Figure 1. From the slope (5.52×10^3 $eq^{-1} \cdot cm^{-1}$) of the plots, the immobilization yield (γ) of **1a** on NH_2–SiO_2 was estimated to be 23.8%.[7] Thus, the loading amount (*x* mol/g) of SbTPP chromophore in 1 g of SiO_2 can be calculated using $x = 2.53 \times 10^{-3} \gamma \cdot a$.

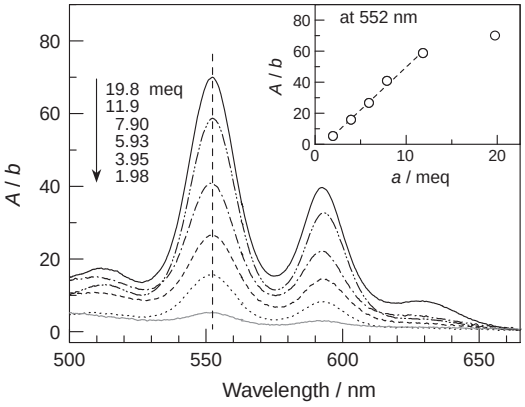


Figure 1. Absorption spectra of a bead of **2** in various *a* values. Inset shows the plots of *A*/*b* vs the *a* value.

The fluorescence spectra of a bead of **2** were remarkably different from that of **1b** in solution; new broad emission appeared near 630 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 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 meq of SbTPP chromophore

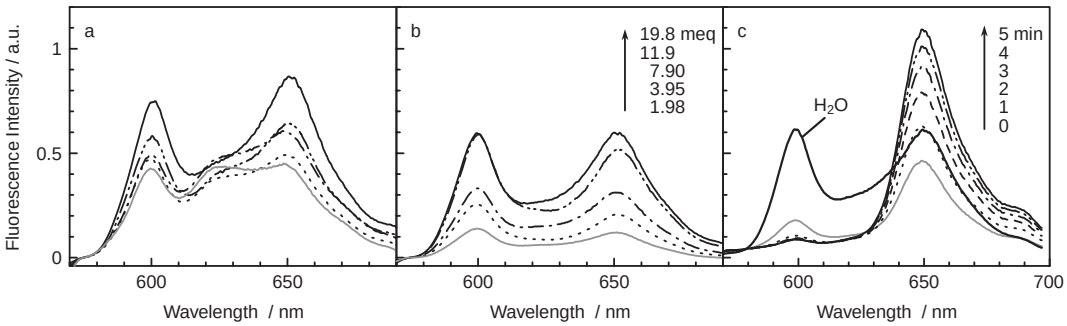


Figure 2. Fluorescence spectra of a bead of **2** (a) before and (b) after acetylation. (c) Fluorescence spectral change of a bead of the acetylated **2** in MeCN solution of 5 mmol/dm^3 Et_2NH under irradiation of a laser light at 543 nm.

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 NH_2–SiO_2, the composition ratio, SiO_2:HO–Si–$C_3H_6NH_2$, is calculated to be 6.9:1. If the SiO_2 moieties were arranged in two dimensions, the aminopropyl groups presumed to be apart from 2–3 units of SiO_2 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 Et_2NH (5 mmol/dm^3) was introduced with microsyringe pump into the MCR, where beads of the acetylated **2** ($x = 7.17$ μmol/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 (H_2TPPCONH–SiO_2) 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 Et_2NH 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 Et_2NH ($E_{1/2\ ox} = 1.01$ V vs Ag/Ag^+)[10] and the reduction potentials and the excitation energy of SbTPP chromophore.[11] Therefore, the photoinduced electron transfer from Et_2NH 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 powerful tool for the analysis and photochemical reaction in microregion on beads.

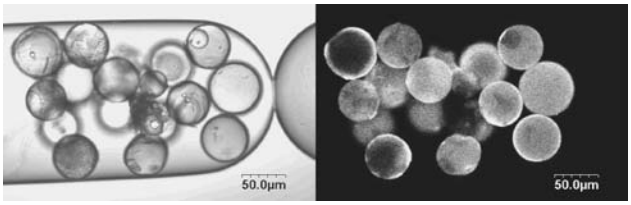


Figure 3. Microscopic image of the acetylated **2** in a MCR (left) and its CLSM fluorescence image (right).

This work was supported by a Grand-in-Aid for Scientific Research on Priority Areas (417) from Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japan Government.

References and Notes

1 D. Gust and T. A. Moore, in "The Porphyrin Handbook," ed. by K. M. Kadish, K. M. Smith, and R. Guilard, Academic Press, New York (2000), Vol. 8, p 153.
2 T. Shiragami, Y. Shimizu, K. Hinoue, Y. Fueta, K. Nobuhara, I. Akazaki, and M. Yauda, *J. Photochem. Photobiol., A*, **156**, 115 (2003).
3 T. Shiragami, R. Makise, Y. Inokuchi, J. Matsumoto, H. Inoue, and M. Yasuda, *Chem. Lett.*, **33**, 736 (2004).
4 H. Yokoi, T. Shiragami, J. Hirose, T. Kawauchi, K. Hinoue, Y. Fueda, K. Nobuhara, I. Akazaki, and M. Yasuda, *World J. Microbiol. Biotechnol.*, **19**, 559 (2003).
5 C. E. Kibbey and M. E. Meyerhoff, *Anal. Chem.*, **65**, 2189 (1993).
6 Aminopropyl silica gel (NH_2–SiO_2): Fuji Silysia, Average diameter (μm): 56.7, Area (m^2/g): 272, Density (g/mL): 0.62, Content of NH_2 (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 SiO_2. If the mol absorptivity (ε) of a bead of **2** is equal to that of **1b** (1.48×10^4 dm^3·mol^{-1}·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 NH_2–SiO_2. Therefore, the slope of Figure 1 can be represented by Eq 3, resulting that γ is 23.8%. $A = \varepsilon bc$ (Eq 1), $A/b = \varepsilon c = 1.57\varepsilon\gamma a$ (Eq 2) where $c = 1000Wx/V = 2.53W\gamma a/V = 1.57\gamma a$, $x = 2.53 \times 10^{-3}\gamma a$, and $W = 0.62V$. Slope $= 1.57\varepsilon\gamma$ (Eq 3), and $\gamma =$ slope$/(1.57\varepsilon)$.
8 K. Sato, M. Tokeshi, H. Kimura, and T. Kitamori, *Anal. Chem.*, **73**, 1213 (2001).
9 G. H. Seong and R. M. Crooks, *J. Am. Chem. Soc.*, **124**, 13360 (2002).
10 C. K. Mann, *Anal. Chem.*, **36**, 2424 (1964).
11 Y. Andou, T. Shiragami, K. Shima, and M. Yasuda, *J. Photochem. Photobiol., A*, **147**, 191 (2001). $E_{1/2\ red}$ vs. $Ag/Ag^+ = -0.73$ V for $[Sb(OH)_2TPP]PF_6$ and $E_{0\text{–}0} =$ 2.08 V for **1b**.

Published on the web (Advance View) September 28, 2005; DOI 10.1246/cl.2005.1484