Effective Inclusion of Chlorophyllous Pigments into Mesoporous Silica Modified with α,ω-Diols

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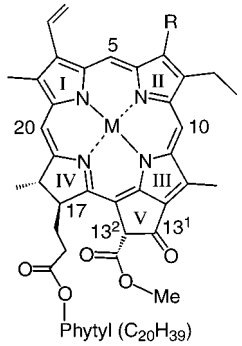
FSM-type mesoporous silica modified with various diols was utilized as an inorganic medium for immobilizing chlorophyll *a* (Chl *a*) without denaturation. The energy transfer from Chl *b* to Chl *a* in the mesopores was also observed for the first time. Silanol groups on the mesoporous silica were esterified with diols, and this was confirmed by ^{13}C MAS NMR, IR, N_2 adsorption, and elemental analysis. The suppression of the pigment degradation in modified FSM was confirmed by both visible absorption spectroscopy of the FSM/Chl compounds and the analysis of the extracts from FSM/Chl by high-performance liquid chromatography. The fluorescence emission bands of Chl *a* and Chl *b* in FSM modified with 1,6-hexanediol appeared at 672 and 654 nm, respectively. In the FSM/Chl containing both Chl *a* and Chl *b*, the emission band due to Chl *b* was decreased whereas the band due to Chl *a* was increased. This is in sharp contrast to the emission spectrum of an acetone solution containing both of Chls, implying energy transfer from Chl *b* to Chl *a* in modified mesoporous silica.

Introduction

The primary part of the photosynthesis process is driven by a light-induced redox reaction in a reaction center complex, and sunlight is collected by antenna complexes. In the redox reaction, energy is efficiently transferred to a specific site (primary donor) to generate a high-energy electron.[1,2] The primary processes are driven by chlorophylls (Chls, Figure 1) and bacteriochlorophylls. It is of general interest to construct artificial photoredox systems for conversion and storage of solar energy as an alternative to using fossil fuels.[3,4] To this end, two basic approaches have emerged. One is to build up supramolecules such as multiporphyrin arrays whose chromophores are fixed by covalent bonds.[5] Although these supramolecules exhibit high efficiency in energy and electron transfers, it is very difficult to design and synthesize these supramolecules, indicating that this strategy is not always attractive for mimicking natural photosynthesis. The other approach is to use adequate media for immobilization of chlorophyllous pigments. Since biofunctional molecules are more easily obtained than those described by the former method, there have been many reports that deal with the energy transfer between chlorophyllous pigments. These occur not only in various solutions[6] but also in various immobilization media such as surfactant micelles,[7] liquid crystals,[8] lipid vesicles and bilayers,[9] LB films,[10] polymer films,[11] and cast films.[12] However, little is known about appropriate inorganic media, though they



Chl *a* : M = Mg, R = Me
Chl *b* : M = Mg, R = CHO
Pheo *a* : M = 2H, R = Me

Figure 1. Molecular structure of chlorophyllous pigments with partial numbering according to the IUPAC system: Chl *a*, R = Me, M = Mg; Chl *b*, R = CHO, M = Mg; Pheo *a*, R = Me, M = 2H.

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(1) Pullerits, T.; Sundström, V. *Acc. Chem. Res.* **1996**, *29*, 381.
(2) Cogdell, R. J.; Lindsay, J. G. *TIBTECH* **1998**, *16*, 521.
(3) Gust, D.; Moore, T. A.; Moore, A. L. *Acc. Chem. Res.* **1993**, *26*, 198.
(4) Dutta, P. R.; Ledney, M. *Prog. Inorg. Chem.* **1997**, *44*, 209.
(5) (a) Wagner, R. W.; Lindsey, J. S. *J. Am. Chem. Soc.* **1994**, *116*, 9759. (b) Hsiao, J.-S.; Krueger, B. P.; Wagner, R. W.; Johnson, T. E.; Delaney, J. K.; Mauzerall, D. C.; Fleming, G. R.; Lindsey, J. S.; Bocian, D. F.; Donohoe, R. J. *J. Am. Chem. Soc.* **1996**, *118*, 11181. (c) Nakano, A.; Osuka, A.; Yamazaki, I.; Yamazaki, T.; Nishimura, Y. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 3023. (d) Luo, C.; Guldi, D. M.; Imahori, H.; Tamaki, K.; Sakata, Y. *J. Am. Chem. Soc.* **2000**, *122*, 6535. (e) Lammi, R. K.; Ambroise, A.; Balasubramanian, T.; Wagner, R. W.;. Bocian, D. F.; Holten, D.; Lindsey, J. S. *J. Am. Chem. Soc.* **2000**, *122*, 7579.
(6) (a) Frackowiak, D.; Wróbel, D. *Biophys. Chem.* **1973**, *1*, 125. (b) Bauer, R. K.; Szalay, L.; Tombacz, E. *Biophys. J.* **1972**, *12*, 731. (c) Losev, A. P.; Zen'kevich, E. I. *Zh. Prikl. Spektrosk.* **1968**, *9*, 144. (d) Watson, W. F.; Livingston, R. *J. Chem. Phys.* **1950**, *18*, 802.

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are very suitable for applications in energy conversion and storage. We have recently proposed the use of mesoporous silica as the adsorbents of Chls.[13,14]

Ordered mesoporous silicas possess both high surface areas with ordered mesopores and large adsorption capacities,[15] which make them useful as catalysts, catalyst supports, and adsorbents for larger molecules.[16] The advantages of mesoporous silica in comparison with conventional media are as follows. (1) The high surface area (ca. 1000 $m^2\ g^{-1}$) of mesoporous silica is suitable for increasing the amount of adsorbed pigments per unit volume. This assumes that most of immobilized pigments are inclined to be in a monomeric state in the adsorbents. As such, they have higher fluorescence quantum yields than aggregates. It would also facilitate the spectral analyses because the systems would contain only monomeric species. (2) To decrease the dissipation of energy, chlorophyllous pigments in living organisms are in close contact with surrounding proteins.[17] Here we point out the possibility that immobilized Chls are fixed in the mesopores, with very little diffusion. Such immobilization is appropriate for rapid energy transfer with high efficiency as in protein matrices. (3) Silica pigment complexes without solvents are suitable for simple handling. These systems also suppress pigment denaturation because chlorophyllous pigments in solid states are less prone to be denatured than those in solutions. (4) Extended applications of mesoporous silica have been proposed by using surface modification (such as esterification and silylation) of silanol (Si−OH) groups in mesopores.[18] The surface modification should create a variety of applications of mesoporous materials because they retain their desirable features, including mechanical strength.

In a previous paper, we reported the adsorption of Chl *a* into FSM-type mesoporous silica derived from a layered polysilicate kanemite.[13] However, almost all of the Chl *a* was pheophytinized (demetalation of Chl) after adsorption due to interactions with acidic silanol groups on the silica surface. Pheophytinization of Chl *a* was appreciably suppressed by use of FSM modified with 1,4-butanediol[13] or by incorporation of transmetalated Chls such as Zn−Chl *a* into bare FSM.[14] Nevertheless, it is well-known that chlorophyllous pigments are labile to heat or solvent treatments, resulting in pheophytinization and allomerization (oxidation of ring V).[19] Furukawa et al. reported that Zn−Chl *a* was mostly allomerized during the adsorption, though such denaturation is not detectable by visible absorption spectra.[14] In this context, the estimation of a variety of Chl denaturations in immobilized media is still an essential subject to be tackled before construction of artificial photosynthetic models.

There are only a few works detailing pigment denaturation in immobilized media (an exception is LB films).[20] The reasons why the integrity of Chls have not been examined in more detail are (1) the lack of the adequate procedures for pigments separation and analysis by high-performance liquid chromatography (HPLC) and (2) the difficulty in the extraction of pigments from immobilized media. In this report, we describe the synthesis of useful inorganic adsorbents that suppress denaturation of Chls. The validity of FSM modified with diols was estimated from the degree of denaturation by use of normal phase HPLC analysis. Furthermore, the occurrence of the energy transfer from Chl *b* to Chl *a* in mesopores was investigated by fluorescence emission for the first time. A series of results provide valuable insight into the design of silica/organic materials for artificial photosynthesis systems.

Experimental Section

Materials. High-purity water glass (SiO_2 = 21.12%, Na_2O = 6.57%, Nippon Chemical Ind. Co.) was used as a silica source. Reagent-grade 1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, acetone, and toluene were purchased from Kanto Chemical Co. HPLC-grade hexane, methanol, 2-propanol, and acetone (Wako) were used for HPLC analysis. Reagent grade hexane and acetone (Wako) were also used for preparative HPLC.

Synthesis of Mesoporous Silica and Surface Modification. FSM-type mesoporous silica (FSM) was prepared by the following method.[13,15e,21] Kanemite was prepared by dispersing 5 g of δ-$Na_2Si_2O_5$ in 100 mL of water, followed by stirring for 30 min. The wet kanemite was dispersed in 100 mL of a 0.1 M (1 M = 1 mol dm^{-3}) octadecyltrimethylammonium chloride

(7) (a) Lehoczki, E.; Csatorday, K.; Szalay, L.; Szebad, J. *Biophysics* **1975**, *20*, 44. (b) Csatorday, K.; Lehoczki, E.; Szalay, L. *Biochim. Biophys. Acta* **1975**, *376*, 268. (c) Chauvet, J.-P.; Bazin, M.; Santus, R. *Photochem. Photobiol.* **1985**, *41*, 83. (d) Jiquan, Z.; Jinhquan, Z.; Jie, X.; Jianping, Z.; Lijin, J. *Colloids Surf. B* **1998**, *11*, 9.

(8) (a) Frackowiak, D.; Zelent, B.; Malak, H.; Cegielski, R.; Goc, J.; Niedbalska, M.; Ptak, A. *Biophys. Chem.* **1995**, *54*, 95. (b) Frackowiak, D.; Szurkowski, J.; Hotchandani, S.; Leblanc, R. M. *Mol. Cryst. Liq. Cryst.* **1984**, *111*, 359.

(9) (a) Borisov, A. Y.; Galutva, O. A.; Godik, V. I.; Mamleeva, N. A. *Mol. Biol.* **1986**, *20*, 1360. (b) Strauss, G.; Tien, H. T. *Photochem. Photobiol.* **1973**, *17*, 425. (c) Colbow, K. *Biochim. Biophys. Acta* **1973**, *314*, 320.

(10) (a) Trosper, T.; Park, R. B.; Sauer, K. *Photochem. Photobiol.* **1968**, *7*, 451. (b) Tweet, A. G.; Bellamy, W. D.; Gaines, G. L., Jr. *J. Chem. Phys.* **1964**, *41*, 2068.

(11) (a) Šiffel, P.; Vavřinec, E. *Photosynthetica* **1980**, *14*, 477. (b) Seely, G. R. *J. Phys. Chem.* **1970**, *74*, 219.

(12) (a) Kelly, R.; Porter, G. *Proc. R. Soc. London A* **1970**, *315*, 149. (b) Sineshchekov, V. A.; Litvin, F. F.; Das, M. *Photochem. Photobiol.* **1972**, *15*, 187. (c) Mamleeva, N. A.; Kropacheva, T. N.; Mitrofanova, A. N.; Pryakhin, A. N. *Russ. J. Phys. Chem.* **1969**, *73*, 1121. (d) Mamleeva, N. A.; Mitrofanova, A. N.; Kropacheva, T. N. *Russ. J. Phys. Chem.* **1997**, *71*, 11007. (e) Kropacheva, T. N.; Mamleeva, N. A. *Russ. J. Phys. Chem.* **1994**, *68*, 609.

(13) Murata, S.; Hata, H.; Kimura, T.; Shgahara, Y.; Kuroda, K. *Langmuir* **2000**, *16*, 7106.

(14) Furukawa, H.; Kuroda, K.; Watanabe, T. *Chem. Lett.* **2000**, 1256.

(15) (a) Yanagisawa, T.; Shimizu, T.; Kuroda, K.; Kato, C. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 988. (b) Kresge, C. T.; Leonowicz, M. E.; Roth, W. J.; Vartuli, J. C.; Beck, J. S. *Nature* **1992**, *359*, 710. (c) Beck, J. S.; Vartuli, J. C.; Roth, W. J.; Leonowicz, M. E.; Kresge, C. T.; Schmitt, K. D.; Chu, C. T.-W.; Olson, D. H.; Sheppard, E. W.; McCullen, S. B.; Higgins, J. B.; Schlenker, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 10834. (d) Inagaki, S.; Fukushima, Y.; Kuroda, K. *J. Chem. Soc., Chem. Commun.* **1993**, 680. (e) Inagaki, S.; Koiwai, A.; Suzuki, N.; Fukushima, Y.; Kuroda, K. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1449.

(16) Corma, A. *Chem. Rev.* **1997**, *97*, 2373.

(17) (a) Kühlbrandt, W.; Wang, D. N.; Fujiyoshi, Y. *Nature*, **1994**, *367*, 614. (b) McDermott, G.; Prince, S. M.; Freer, A. A.; Lawless, A. M. H.; Papiz, M. Z.; Cogdell, R. J.; Isaacs, N. W. *Nature* **1995**, *374*, 517. (c) Krauss, N.; Schubert, W. D.; Klukas, O.; Fromme, P.; Witt, H. T.; Saenger, W. *Nat. Struct. Biol.* **1996**, *3*, 965.

(18) (a) Stein, A.; Melde, B. J.; Schroden, R. C. *Adv. Mater.* **2000**, *12*, 1403. (b) Kimura, T.; Saeki, S.; Sugahara, Y.; Kuroda, K. *Langmuir* **1999**, *15*, 2794. (c) Kimura, T.; Kuroda, K.; Sugahara, Y.; Kuroda, K. *J. Porous Mater.* **1998**, *5*, 127.

(19) Schaber, P. M.; Hunt, J. E.; Fries, R.; Katz, J. J. *J. Chromatogr.* **1984**, *316*, 25.

(20) (a) Mingotaud, C.; Chauvet, J.-P.; Patterson, L. K. *J. Phys. Chem.* **1996**, *100*, 18554. (b) Sato, H.; Ozaki, Y.; Oishi, Y.; Kuramori, M.; Suehiro, K.; Nakashima, K.; Uehara, K.; Iriyama, K. *Langmuir* **1997**, *13*, 4676. (c) Sato, H.; Oishi, Y.; Kuramori, M.; Suehiro, K.; Kobayashi, M.; Uehara, K.; Araki, T.; Iriyama, K.; Ozaki, Y. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 621.

(21) Hata, H.; Saeki, S.; Kimura, T.; Sugahara, Y.; Kuroda, K. *Chem. Mater.* **1999**, *11*, 1110.

($[C_{18}H_{37}N(CH_3)_3]Cl$, Tokyo Kasei) solution. After the suspension was stirred at 70 °C for 3 h, the pH was adjusted to 8.5 by adding 2 M HCl and the stirring was continued at 70 °C for 3 h. The solid product was recovered, washed with water, air-dried, and calcined at 550 °C for 6 h in air.

The reaction of FSM-type mesoporous silica with diols was performed as follows.[13] FSM was pretreated at 150 °C for 3 h in vacuo to remove adsorbed water. The dried FSM (1.0 g) and 75 mL of 1,4-butanediol (BD), 1,6-hexanediol (HD), or 1,8-octanediol (OD) were refluxed for 24 h under an N_2 atmosphere at 230, 250, or 280 °C, respectively. The esterified products (BD-FSM, HD-FSM, and OD-FSM) were washed twice with acetone to remove unreacted diols and dried at room temperature in vacuo.

Preparation and Adsorption of Chls. The preparation of pure Chl *a* and *b* was described elsewhere.[22,23] Briefly, chlorophyllous pigments were extracted from lyophilized spinach leaf tissues, and purified using a preparative normal-phase HPLC (column: Senshupak Silica-5251N, 20 mm diameter × 250 mm, 4 °C; eluent: hexane/acetone/methanol = 100/5/1.5 for Chl *a*; hexane/2-propanol = 100/3 for Chl *b*). The adsorption of Chl *a* and Chl *b* by FSM modified with diols was performed as follows:[13] a prescribed amount of Chl was dissolved in 5 mL of toluene, and then 0.1 g of FSM modified with a diol was dispersed in the Chl solution. The mixture was stirred at room temperature for 3 h in the dark and centrifuged to remove supernatant and dried at room temperature in vacuo. Chlorophyllous pigments adsorbed on bare FSM and modified FSM were extracted with acetone and injected onto an analytical HPLC column to determine the degree of pigment degradation (column = Senshupak Silica-1251N 4.6 mm diameter × 250 mm, 4 °C; the eluents were the same as for preparative HPLC; integrator, Shimadzu C−R6A). The Chl *a*/Pheo *a* molar ratio was deduced from calibration curves as described previously.[22,24]

Instrumentation. X-ray powder diffraction (XRD) patterns were obtained with a Mac Science M03XHF[22] diffractometer (Mn-filtered Fe Kα radiation). The nitrogen adsorption−desorption isotherms were measured at 77 K in the relative pressure range from about 10^{-5} to 0.99 on a Belsorp 28SA instrument (Japan Bell Inc.). The pore size distributions were calculated from the adsorption data by the BJH method.[25] The BET surface areas were determined by using the data under the relative pressures between 0.05 and 0.25 before the capillary condensation. The pore volumes were obtained by the *t*-plot method. Infrared spectra were recorded on a Perkin-Elmer FTIR 1640 by Nujol and KBr disk techniques. For the measurements of Nujol technique, the esterified products were preheated at 120 °C for 3 h to remove adsorbed water. Solid-state ^{13}C magic angle spinning (MAS) NMR measurements were performed on a JEOL JMM-CMX400 spectrometer at a spinning rate of 10 kHz and a resonance frequency of 100.54 MHz with a pulse length of 4.0 μs and a recycle time of 60 s. The chemical shifts were expressed with respect to hexamethylbenzene. Solid-state ^{29}Si MAS NMR measurements were performed at a spinning rate of 3.5 kHz and a resonance frequency of 79.42 MHz with a 45° pulse length of 2.0 μs and a recycle time of 100 s. The ^{13}C and ^{29}Si MAS NMR spectra were deconvoluted with the program package IGOR Pro Ver. 3.1 (WaveMatrix, Inc.). Proton NMR spectra ($CDCl_3$ solutions) were recorded on a 270 MHz spectrometer (JEOL JNM-EX270). Differential thermal analysis (DTA) curves were obtained with a Mac Science 2000S analyzer at a heating rate of 10 °C min^{-1} under a dry air flow (standard: α-Al_2O_3). Elemental analysis (CHN) was performed on a Perkin-Elmer PE−2400II. Diffuse reflectance visible absorption and fluorescence spectra were recorded on a Shimadzu spectrophotometer (UV-3101PC) and a Hitachi spectrofluorophotometer (F-4500), respectively. The fluorescence spectra were corrected using an attachment of standard tungsten lamp (Hitachi).

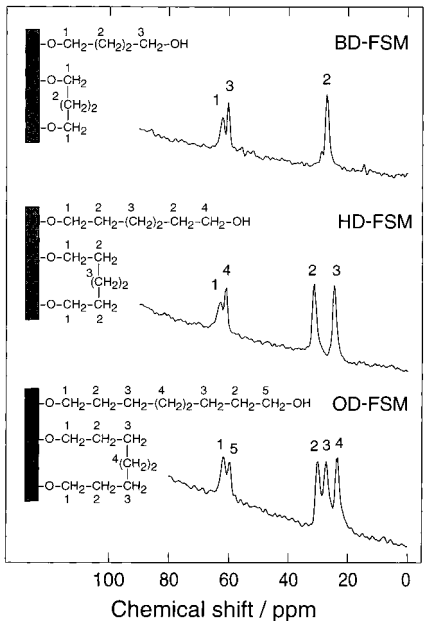

Figure 2. Solid-state ^{13}C MAS NMR spectra of esterified FSM.

Results

Characterization of FSM Modified with Diols. The XRD pattern of FSM had four peaks at *d*-spacings of 3.9, 2.3, 2.0, and 1.5 nm (see Supporting Information, Figure S1), which are characteristic of a typical hexagonal array of mesopores.[15e] The XRD patterns of diol-modified FSM (BD-, HD-, and OD-FSM) were quite similar to that observed for unmodified FSM, indicating the retention of the structure after the esterification.

Esterified FSM powders were characterized by ^{13}C MAS NMR, FT-IR, DTA, and N_2 adsorption analyses. The ^{13}C MAS NMR spectra of diol-modified FSM are shown in Figure 2. All the signals were assigned to corresponding carbon atoms in 1,4-butanediol, 1,6-hexanediol, and 1,8-octanediol, indicating the presence of $-O(CH_2)_4O-$, $-O(CH_2)_6O-$, and $-O(CH_2)_8O-$ groups, respectively.[26] Two kinds of carbon atoms bonded to oxygen were detected at around 61 ppm, showing that one or two OH groups in diols were linked to the surface silanol groups of FSM. The ratio of diols having both OH groups esterified with silanol groups (the bridged type) increased with an increase in the alkylene chain length for diol, that is, in the order BD-, HD-, and OD-FSM. The ratios of the terminal carbons of one side and both sides in the diols used for esterification were estimated to be 99:1, 81:19, and 65:35 for BD-, HD-, and OD-FSM, respectively.

Surface modification of FSM was also confirmed by the DTA curves (see Supporting Information, Figure S2). Sharp exothermic peaks appeared at 282, 272, and 267 °C in the curves of BD-, HD-, and OD-FSM, respectively. These DTA curves were reminiscent of the

(22) Watanabe, T.; Hongu, A.; Honda. K.; Nakazato, M.; Konno, M.; Saitoh, S. *Anal. Chem.* **1984**, *56*, 251.
(23) Furukawa, H.; Oba, T.; Tamiaki, H.; Watanabe, T. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 1341.
(24) Oba, T.; Kobayashi, M.; Yoshida, S.; Watanabe, T. *Anal. Sci.* **1996**, *12*, 281.
(25) Barrett, E. P.; Joyner, L. G.; Halenda, P. P. *J. Am. Chem. Soc.* **1951**, *73*, 373.
(26) Tuel, A.; Hommel, H.; Legrand, A. P.; Balard, H.; Sidqi, M.; Papirer, E. *Colloids Surf.* **1991**, *58*, 17.

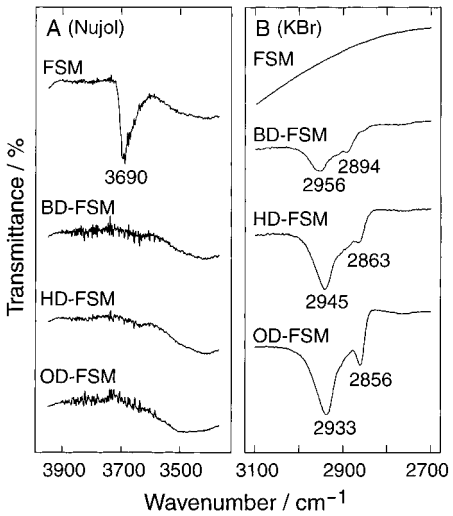

Figure 3. FT-IR spectra of unmodified FSM and esterified FSM by (A) Nujol and (B) KBr disk techniques.

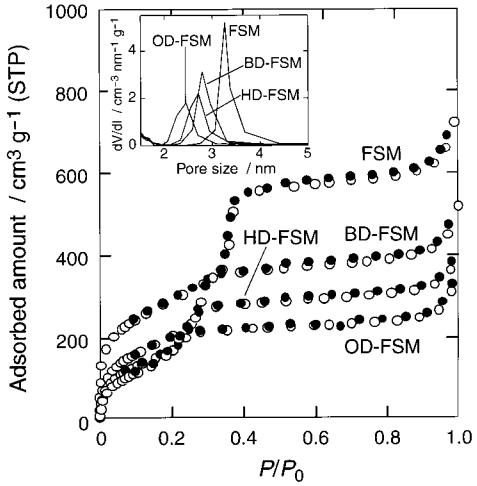

Figure 4. N_2 adsorption−desorption isotherms of unmodified FSM and esterified FSM: open symbols, adsorption; filled symbols, desorption. Inset: Corresponding pore size distribution obtained from the adsorption branch by the BJH method.

exothermic peaks of FSM modified with *n*-alcohols,[18c] indicating the combustion of the esterified organic groups.

Figure 3 shows the IR spectra of unmodified and modified FSM. In the IR spectrum of unmodified FSM obtained by the Nujol technique (Figure 3A), the band due to ν(OH) of Si−OH groups was observed at 3690 cm^{-1}.[18b] This peak all but disappears after the reaction with diols. The bands at around 2900 cm^{-1} due to the vibrations of C−H bonds were observed for modified FSM by the KBr disk technique (Figure 3B)[27] and these bands increased from BD-FSM to OD-FSM. These findings indicate that most of the Si−OH groups on FSM surfaces reacted with diols to form 1-hydroxyalkoxide groups.

The N_2 adsorption isotherms of esterified FSM and the pore size distributions estimated by the BJH method are illustrated in Figure 4. All of the N_2 adsorption isotherms are type IV, indicating the presence of mesopores after the surface modification. This trend is

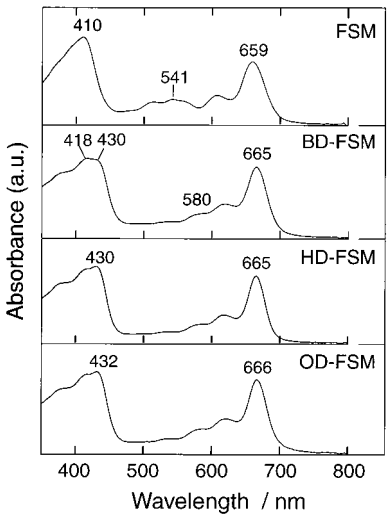

Figure 5. Diffuse reflectance visible absorption spectra of Chl *a* adsorbed on unmodified FSM and esterified FSM. (Note: the absorption maxima of Pheo *a* in toluene were 415 (Soret), 538 (Q_x), and 672 nm (Q_y), and those of Chl *a* were 433 (Soret), 580 (Q_x), and 665 nm (Q_y). See the text for the details.)

Table 1. Pore Characterization of Unmodified FSM and Esterified FSMs and Grafted Numbers of the Organic Groups in the Esterified FSMs

	BET surface area ($m^2 \cdot g^{-1}$)	pore vol ($mL \cdot g^{-1}$)	pore size (nm)	no. of grafted groups ($groups \cdot nm^{-2}$)
FSM	1000	0.82	3.2	-
BD-FSM	760	0.63	2.8	2.4
HD-FSM	610	0.42	2.7	2.0
OD-FSM	540	0.32	2.4	2.0

similar to that reported previously for the adsorption isotherms of mesoporous silicas before[15e] and after modification.[18c] The BET surface areas and the pore volumes of modified FSM decreased with the alkylene chain length of the diols (Table 1). The full widths at half-maximum of the pore size distributions were narrow in all the samples, and no drastic changes were detected by the modification. It should be noted that the pore size of these FSM decreased with an increase in the alkylene chain length (Figure 4 inset and Table 1).

Visible Absorption Spectra of Adsorbed Chl *a*. The Q_y, Q_x, and Soret absorption peaks of a monomeric Chl *a* solution containing 100 nmol of Chl *a* are located at 665, 580, and 433 nm, respectively (in toluene, data not shown). Modified and unmodified FSM were dispersed in the Chl solutions described in the Experimental Section. The supernatants became almost colorless after several minutes. Considering both the decrease in the absorption of the supernatant solutions containing Chls and the large inner surface area of FSM in comparison with outer surface area,[21] it is assumed that most of the Chls were introduced into the pore system of FSM.[28]

Surface modification of FSM considerably affects the spectral shape of the absorption spectra (Figure 5). The Q_y, Q_x, and Soret absorption peaks of Chl on bare FSM were located at 659, 541, and 410 nm, respectively. There are several differences from the spectrum of Chl

(27) Burkert, S. L.; Sims, S. D.; Mann, S. *Chem. Commun.* **1996**, 1367.

(28) Because the amount of adsorbed Chl was so small, there was some uncertainty in the determination of the amount of incorporated Chl by the N_2 adsorption data.

a in toluene. The peak positions in the Soret and Q_x regions were similar to those of Pheo *a* in toluene due to pheophytinization of adsorbed Chl (Soret, 415 nm; Q_x, 538 nm; Q_y, 672 nm for Pheo *a* in toluene). The Q_y peak position of FSM/Chl was located at shorter wavelength than that of Pheo *a* in toluene, because of the partial protonation of Pheo *a*.[29] These findings indicate that most of incorporated Chl were pheophytinized in the adsorption process. This is reminiscent of the absorption spectrum of bare FSM containing pure Pheo *a* with a Soret band at 406 nm.[14] The ^{1}H NMR spectrum of the pigments extracted from bare FSM showed the peak due to the N−H group in Pheo *a* at −1.9 ppm (data not shown).[30]

On the other hand, Chl *a* incorporated into the modified FSM exhibited an absorption spectrum similar to that of Chl *a* in toluene with Q_y and Q_x peaks at around 665 and 580 nm, respectively. There was no absorption band assigned to the Q_x transition of Pheo *a* at around 540 nm. When HD- and OD-FSM were used, the absorption peaks in the Soret region appeared at around 430 nm. These spectral shapes are similar to the spectrum of Chl *a* in toluene, indicating that pheophytinization was effectively suppressed by the surface modification with diols. However, BD-FSM/Chl gave two Soret absorption peaks at 418 and 430 nm, which suggests partial pheophytinization of Chl *a* in this system.

The Q_y peaks of Chl on surface modified FSM were broadened in comparison to that of Chl *a* in toluene (full width at half-maximum of Q_y band: 840 cm^{-1} in HD-FSM; 400 cm^{-1} in toluene). Similar tendencies were reported for clay/Chl and FSM/Zn−Chl systems.[14,31] Although aggregate species generally gave broadened absorption peaks,[32,33] the broad Q_y band observed here probably resulted from a single species, because the second derivative of the absorption spectrum in the Q_y region yielded a curve with one minimum. Consequently, almost all Chl was in a monomeric state in the pore system.[14]

HPLC Analyses. HPLC analyses of chlorophyllous pigments extracted from the FSM/Chl system were performed to estimate the degree of pigment degradation (Figure 6). The extracted pigments were classified into four categories: (1) Chl *a*, (2) Chl *a*′ (C13^2-(*S*)-epimer of Chl *a*),[22] (3) Pheo *a*/*a*′ (demetalated compounds of Chl *a*/*a*′), and (4) allomers (oxidation of ring V of Chls).[19] Because Chl *a* ⇄ Chl *a*′ epimerization is reversible stereoisomerization at the C13^2 moiety, Chl *a* isomerizes to Chl *a*′ in solutions, especially under the presence of a base catalyst.[34] Because the visible absorption and fluorescence spectra and redox properties of Chl *a* and *a*′ are almost the same due to the common π-conjugated system,[22,23,35] in this report, we regard pheophytinization and allomerization as denaturation of Chl *a* during the adsorption process.

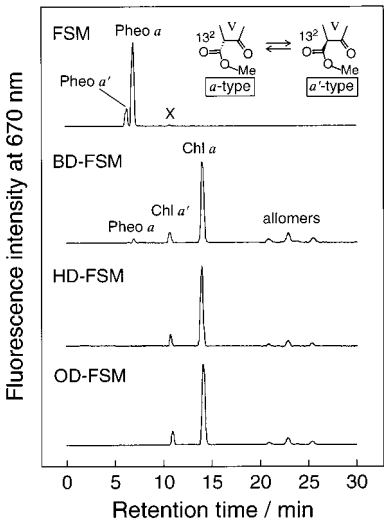

Figure 6. HPLC traces of Chls extracted from unmodified FSM and esterified FSM. The unidentified minor peak at 11 min (top trace) may be due to a pheophytin allomer.

Almost all the Chl *a* molecules changed to Pheo *a*/*a*′ when unmodified FSM was used (Figure 6 top). On the other hand, pheophytinization of Chl *a* was effectively suppressed by the surface modification of FSM with diols. Though the composition of the pigments extracted from BD-FSM showed small demetalation of Chl (ca. 5%), no Pheo derivatives were detected for the extracts from HD- and OD-FSM. The traces for the diol-modified FSM also revealed the formation of allomers of Chl *a* in the pore systems, being consistent with the view that Chls are liable to be allomerized.[36] The ratio of allomerization of Chl was suppressed to ca. 20%, indicating that a large part of Chl *a* was not denatured within diol-modified FSM. This is in sharp contrast to the HPLC trace for Zn−Chl *a* extracted from naked FSM (approximately 90% of Zn−Chl *a* was allomerized).[14]

Coadsorption of Chl *a* and *b* into Diol-Modified FSM. As proved clearly in the above subsection, surface modification of FSM with diols is quite useful to suppress the degradation of Chls. It is very intriguing to mimic some functions of photosynthesis, such as energy transfer from Chl *b* to Chl *a* in native light-harvesting antenna complex II (LHC II).[17a] To this end Chl *a* and Chl *b* were coadsorbed onto the HD-FSM pore system (the amounts of Chls added were Chl *a*/*b* = 50/0, 0/5, and 50/5 nmol). The difference in the absorption spectra of the supernatant solutions before and after the adsorption indicates that roughly 90% of Chls were incorporated into the HD-FSM mesopores. The result means that the amount of immobilized Chls in the coadsorbed system (Chl *a*/*b* = 50/5 mol) was almost same as a simple sum of Chl *a* (Chl *a*/*b* = 50/0 nmol) and Chl *b* (Chl *a*/*b* = 0/5 nmol) into HD-FSM.

Figure 7 depicts the diffuse reflectance visible absorption spectra of Chls in HD-FSM. Chl *a* and Chl *b* gave the Soret peaks at 430 and 459 nm and the Q_y peaks at 665 and 648 nm, respectively (Figure 7, curves a and

(29) The Q_y band of protonated Pheo *a*, was at 654 nm in a HCl/acetone solution (data not shown).

(30) Hynninen, P. H.; Lötjönen, S. *Magn. Reson. Chem.* **1985**, *23*, 605.
(31) Itoh, T.; Ishii, A.; Matsushima, A.; Hiroto, M.; Nishimura, H.; Tsuzuki, T.; Kamachi, T.; Okura, I.; Inada, Y. *Bioconjugate Chem.* **1998**, *9*, 409.
(32) Oba, T.; Mimuro, M.; Wang, Z.-Y.; Nozawa, T.; Yoshida, S.; Watanabe, T. *J. Phys. Chem. B* **1997**, *101*, 3261.
(33) Furukawa, H.; Oba, T.; Tamiaki, H.; Watanabe, T. *J. Phys. Chem. B* **1999**, *103*, 7398.
(34) Watanabe, T.; Mazaki, H.; Nakazato, M. *Biochim. Biophys. Acta* **1987**, *892*, 197.
(35) Hynninen, P. H.; Sievers, G. *Z. Naturforsch.* **1981**, *36B*, 1000.
(36) Mazaki, H. Doctoral thesis, The University of Tokyo, 1990 (in Japanese).

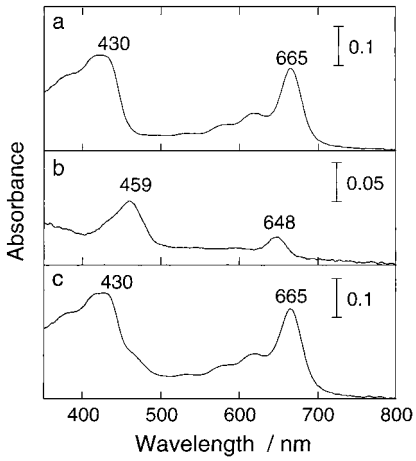

Figure 7. Diffuse reflectance visible absorption spectra of (a) Chl *a*, (b) Chl *b*, and (c) Chl *a*/*b* in FSM modified with 1,6-hexanediol.

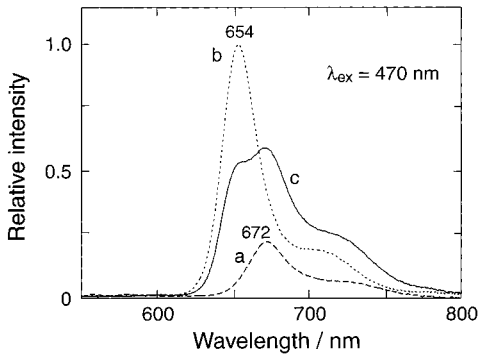

Figure 8. Fluorescence emission spectra of (a) Chl *a*, (b) Chl *b*, and (c) Chl *a*/*b* in HD-FSM (excited at 470 nm).

b). The absorption spectrum of coadsorbed Chls was interpreted as a simple sum of those of neat Chl *a* and Chl *b*. The integrity of Chl *b* was also retained, as determined by analytical HPLC (data not shown).

The fluorescence emission spectra of HD-FSM/Chls are illustrated in Figure 8 (excited at 470 nm). Concentration quenching is negligible in all these HD-FSM/Chls (data not shown). The emission peaks of Chl *a* and Chl *b* on HD-FSM were located at 672 and 654 nm (Q_y-(0−0) transition), respectively, with a small shoulder due to the Q_y(0−1) transition at 710−720 nm (Figure 8, curves a and b).[37] On the other hand, the coadsorbed system (curve c) gave the emission peak at 672 nm with a shoulder at around 650 nm. When the ratio of Chl *a*/*b* was varied at 10/1, 4/1, and 1/1, the emission intensity for 672 nm band decreased with the corresponding increase in the 654 nm band (see Supporting Information, Figure S3). This clearly demonstrates that the 672 and 654 nm emission bands originate from the emission of Chl *a* and Chl *b*, respectively. The peak area originating from the Chl *b* emission band in the coadsorbed system (curve c) was roughly 0.3-fold smaller than that in the pure Chl *b* emission band (curve b), while the fluorescence intensity of the 672 nm band in the coadsorbed system double that of pure Chl *a* (curve a) when the Chl *a*/*b* ratio was 10/1.[38] The findings strongly suggest that Chl *b* was quenched by neighboring Chl *a* and that the excited energy of Chl *b* was transferred to Chl *a*. When the Chl *a*/*b* ratios were 4/1 and 1/1, the peak areas of the emission from Chl *b* were about 0.65 and 0.9 times smaller than that from the single Chl *b* system (see the Supporting Information Figure S3). The results suggest that the energy transfer becomes more difficult as the Chl *a*/*b* ratio decreases.

Fluorescence Emission Spectra of Chls in Acetone. Emission spectra of Chls in acetone were examined as a reference (see Supporting Information, Figure S4, excited at 470 nm). There was no quenching of the emission band at around 651 nm (originated from Chl *b*) under the presence of both Chl *a* and *b*, if compared with the case of excited neat Chl *b*. The emission spectrum of the system is almost the sum of the emission spectra of Chl *a* and Chl *b*.

Discussion

Surface Modification of FSM. The number of grafted diols, calculated based on the CHN data, and the porosities[18c] are listed in Table 1. The ratio of hydroxyl groups from singly vs doubly grafted diols (see Results) should be considered. In addition to esterified OH groups on one side of diols, roughly $^1/_5$ and $^1/_3$ of the OH groups on the other side in 1,6-HD and 1,8-OD were linked to Si−OH groups of FSM, respectively. Accordingly, the coverage of esterified Si−OH groups are estimated to be 2.4, 2.4, and 2.7 groups nm^{-2} for BD-, HD-, and OD-FSM, respectively. These values, however, are slightly lower than the density of silanol groups on the bare FSM surface (2.8 groups nm^{-2}; calculated by the intensity ratio of ^{29}Si MAS NMR signals due to Q^3 and Q^4 Si environments, data not shown). Therefore, some unreacted Si−OH groups exist after the modification of FSM.

As mentioned above, the pore size decreased with the alkylene chain length whereas the full widths at half-maximum of the peaks of the pore size distribution curves hardly changed after the modification (Figure 4 inset). This means that the mesopores are uniform after the modification and that the organic groups cover the mesopores homogeneously. The pore size of FSM (3.2 nm) decreased by 0.4, 0.5, and 0.8 nm after the modification with 1,4-BD, 1,6-HD, and 1,8-OD, respectively (see Table 1). If the alkylene chains of the diols take the all-trans conformation and are directed toward the center of the mesopores, the pore size should decrease by 1.1, 1.7, and 2.2 nm for BD-, HD-, and OD-FSM, respectively. The actual decreases are lower than those values, suggesting that the diols are inclined to some extent to overlap with each other to cover the inner surfaces of FSM.

Denaturation of Chl *a*. Because silanol groups act as acid sites in FSM,[39] the central Mg ions are exchanged with protons when Chls are adsorbed onto bare FSM.[13] Chls are allomerized in a much more facile manner than Pheo.[36] Figure 6 (top) shows that only a very small peak (designated as X), due to allomers of

(37) Petke, J. D.; Maggiora, G. M.; Shipman, L.; Christoffersen, R. E. *Photochem. Photobiol.* **1979**, *30*, 203.

(38) The emission spectrum of coadsorbed Chls in the HD-FSM pore system was approximately interpreted as a summation of the 2-fold Chl *a* emission spectrum and the 0.3-fold Chl *b* one.

(39) Yamamoto, T.; Tanaka, T.; Funabiki, T.; Yoshida, S. *J. Phys. Chem. B* **1998**, *102*, 5830.

Pheo, was observed. Therefore, Chl was pheophytinized before allomerization; that is, the rate of pheophytinization of Chls is much faster than that of allomerization. On the other hand, pheophytinization was not observed when the surface was modified by diols. The fact that pheophytinization was largely suppressed in the diol-modified mesoporous silica means a wide applicability of FSM mesoporous silica as an immobilizing medium for functional and biochemical molecules.

Pheophytinization occurred slightly (~5%) on the adsorption of Chls to BD-FSM, suggesting that unesterified silanol groups are present to some extent after the esterification. When the longer alkylene chains were used, pheophytinization due to residual silanol groups was not observed. Therefore, unreacted silanol groups should be effectively covered by those diols. This supposition is in line with the proposed inclined arrangement of diols in the mesopores.

Many organic substances can be used to modify inner-surface silanol groups in FSM. To cap silanol groups (acidic sites), silylating reagents like chloroalkylsilane are possible to be employed. However, Chls are not adsorbed onto trimethylsilylated FSM.[13] This fact means that hydrogen bondings between Mg in the chlorin ring or C13^1=O groups and the functional groups in the mesopores are required for Chls to be introduced and adsorbed effectively onto the mesopores.[14] Diols used in this study have OH groups esterified with silanol groups and the OH groups at the ω-terminal that can act as hydrogen bonding sites with Chls after esterification. These OH groups at the ω site play a crucial role for immobilizing Chls. This finding also indicates that the organic modifiers for FSM should possess functional groups (−OH, −SH, or $-NH_2$ for example), which can coordinate with Chls.[12c]

Modification with diols suppresses not only pheophytinization but also allomerization, as shown in Figure 6. Allomerization was suppressed to 20% when Chls were adsorbed onto diol-modified FSM. On the other hand, denaturation of chlorophyllous pigments in LB films was not suppressed completely though the preparation conditions can be controlled easily.[20] Indeed, the degree of suppression based on our HPLC results, especially in the cases of HD- and OD-FSM, was superior to that in the LB films reported in ref 20c.

Energy Transfer from Chl *b* to Chl *a*. Comparing the absorption (Chl *a*) and emission (Chl *b*) spectra, the overlap of the donor emission and acceptor absorption is relatively large (Figures 7 and 8). This overlap indicates that the inductive resonance transfer mechanism for the donor−acceptor interaction (Förster type) should occur.[40] The absorption spectrum is made up of the superposition of Chl *a* and Chl *b* (Figure 7c), indicating that the individual Chls do not exhibit any specific interactions in the ground state within the mesopores, which denies the Dexter type energy transfer.[41] Moreover, because esterified diols in mesopores should be regarded as a sort of solvent, any other chemical interactions between diols and Chls should be negligible. Thus, the Förster type energy transfer is expected to dominate here.

Although there are several reports on the energy transfer between Chls in homogeneous media containing Chls with very high concentrations (e.g., 10^{-3} M),[6b,6d,42] it scarcely occurs at the much lower concentration (roughly 10^{-6} M) in homogeneous media where fluorescence intensity is proportional to pigment concentration. However, even at concentrations as low as 10^{-6} M, the energy transfer may occur if Chls are included in surfactant micelles, because Chl molecules are concentrated 10−1000 times higher in micelles.[7a,b] This is ascribed to the difference in the average distance between the pigments.[7a] To induce the energy transfer, chlorophyllous pigments are required to be accommodated in a confined medium. As a result of the incorporation of Chls in HD-FSM, the distance between the pigments in the mesopores is expected to be shorter than that in a solution; thereby, a similar energy transfer was observed for Chls introduced into HD-FSM. On the basis of the pore volume and the amount of Chl introduced, the concentration of Chl in the mesopore is increased to about 10^{-3} M.[43] It is of interest that the fluorescence intensities of the pigments are proportional to the pigment concentrations under such concentrated conditions and that the energy transfer occurs. On the basis of the pore volume, the distance between neighboring Chls is roughly calculated to be about 14 nm. This value is larger than the Förster critical distance (5−8 nm) calculated in the case of various immobilized media.[7a,b,9c,11a,12a] This difference implies the possibility that the dispersibility of chlorophyllous pigments in mesopores is not so high. Other possibilities are that the chromophores assemble to form an island structure, as in the case of LB films,[45] or that they migrate to the pore entrance during the evaporation of solvent.

The energy transfer efficiency between Chl *b* and Chl *a* in HD-FSM is estimated to be ca. 70%.[38] The efficiency in solution is generally low (20−50%),[6a] while Chls in detergent micelles give high energy transfer efficiencies (>80%).[7a,b] Furthermore, in the case of 7.9×10^{-3} M Chls in polystyrene films, efficiencies in excess of 90% were reported.[11a] These efficiencies are influenced not only by the pigment concentration but also by the donor−acceptor ratio. Therefore, it is difficult to compare these energy transfer efficiencies to each other. Taking into account the trend that high energy transfer efficiencies are obtained at the highest pigment concentrations, diol-modified FSM is of great value as the adsorbents for the effective energy transfer, because small amounts of Chls in the mesopores enable the energy transfer to occur. Nevertheless, the efficiencies in the Chl *a*/*b* ratios of 1/1 and 4/1 were not so high as that observed for 10/1. Consequently, the energy transfer efficiency depends on the number of acceptors (Chl *b*-Chl *a* pairs). In fact, in the light-harvesting Chl *a*/*b*-

(40) Förster, T. In *Modern Quantum Chemistry*; Sinanoğlu, O., Ed.; Academic Press: New York, 1965; p 93.
(41) Dexter, D. L. *J. Chem. Phys.* **1953**, *21*, 836.
(42) It should be pointed out that the Chl concentration of 10^{-3} M needed to be much higher to bring about the energy transfer without concentration quenching.
(43) Enrichment of Chl in the mesopore is also supported by HPLC results. Normally epimerization of Chl scarcely proceed in a toluene solution (20 μM) of Chl *a* if base catalysts such as triethylamine are absent.[23,34] However, when the concentration of Chl is increased, Chl itself has its own catalytic action and epimerization of Chl proceeds (self-catalyzed epimerization).[44]
(44) Mazaki, H.; Watanabe, T. *Biochim. Biophys. Acta* **1990**, *1016*, 190.
(45) Tamai, N.; Yamazaki, T.; Yamazaki, I. *Chem. Phys. Lett.* **1988**, *147*, 25.

protein complex (LHC II), Chl *a* is in close contact with Chl *b* for rapid energy transfer.[17a,46] Thus, to induce highly efficient energy transfer, it may be necessary to optimize the orientation as well as distance between the chromophores.[47]

Conclusions

Chls, which tend to be susceptible to various transformations, are successfully immobilized with suppressed denaturation onto mesoporous silica whose inner surfaces are modified with diols. Effective energy transfer from Chl *b* to Chl *a* in this inorganic medium implicates that mesoporous silica FSM modified with diols can act as both an immobilizing medium of high mechanical strength and also as a confined environment similar to those found in biological systems. The present silica/organic mesostructured materials, which appear to play the role of effective concentration of Chl and light-harvesting antenna, provides very useful information on the studies on the development of photosynthetic model systems. Modification of mesoporous silicas with other reagents and the study on the arrangement of Chl are now in progress.

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Supporting Information Available: Figures showing XRD patterns, DTA curves and fluorescence spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org/.

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(46) Van Grondelle, R. *Biochim. Biophys. Acta* **1994**, *1187*, 1.
(47) Van Patten, P. G.; Shreve, A. P.; Lindsey, J. S.; Donohoe, R. J. *J. Phys. Chem. B* **1998**, *102*, 4209.