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Stabilization of chlorophyll *a* in mesoporous silica and its pore size dependence†

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Chlorophyll *a* extracted from natural *Spirulina* was adsorbed by mesoporous silica, FSM. The amount adsorbed depended on the pore size. Few chlorophyll molecules were adsorbed by a usual silica gel and FSMs with pore sizes of less than 2 nm. However, more than 10 wt% of chlorophyll was adsorbed by FSMs with larger pore sizes. Chlorophyll dissolved in benzene was markedly discolored. On the other hand, a chlorophyll–FSM conjugate in water was much more photostable. The stability increases with increasing pore diameter of the FSM in the chlorophyll–FSM conjugate. The absorption maximum is 665 nm for the isolated chlorophyll *a* in benzene. The adsorption on the outer surface of silica gel and the mesoporous silica shifts the absorption maximum to 673 nm, and the adsorption in the mesopores of diameters of more than 2.5 nm shifts to 677 nm, which resembles the case of chlorophyll in natural leaves. We concluded that the photostability is attributable not only to the chlorophyll–FSM interaction but also to chlorophyll–chlorophyll interactions in the nanometer scale spaces.

Introduction

In the layered thylakoid membranes of chloroplasts in intact leaves, chlorophyll molecules bind to proteins to form chlorophyll–protein conjugates in which the chlorophyll–protein support interaction and nano-scale chlorophyll–chlorophyll interactions play important roles in stabilization and physiological functions.[1] Itoh *et al.* have reported that chlorophyll *a* adsorbed onto silicate layers of smectite minerals showed photostable and photocatalyzed properties,[2–4] which demonstrated the importance of the chlorophyll–support interaction. The interaction between the support and the adsorbed molecules or two molecules in the nano-spaces should be interesting for understanding the excellent properties of natural chlorophyll or enzymes. However, the nano-scale chlorophyll–chlorophyll interaction has seldom been reported except for leaves and photosynthetic bacteria.

There is currently interest in mesoporus materials such as MCM (mobile crystalline material)[5] and FSM (folded-sheet mesoporous material)[6] having honeycomb (hexagonal) structures with ordered cylindrical channels of 2–10 nm in diameter. They are potential hosts for the inclusion of bulky organometallic and inorganic complexes, and offer nano-scale spaces for the desired arrangement of functional molecules such as chlorophyll[7] and metalloporphyrin.[8] We have reported that chlorophyll adsorbed in the pores of FSM showed photostable and photocatalyzed properties.[9] Mesoporous silica with uniform, nanometer size pores should be suitable for studying the nano-scale interactions between the chlorophyll molecules.

The present study deals with the properties of chlorophyll conjugated with FSM of different pore sizes, their photostability, adsorption, and adsorption spectra.

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Experimental section

General

Chlorophyll *a* purified from *Spirulina* was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) Silica gel (surface area: 650 $m^2\ g^{-1}$) was supplied by the Fujisirisia Kagaku Co. Ltd. (Aichi, Japan). Mesoporous materials, FSM-10, -12, -14, -16 and -22 materials, with pore diameters of 1.6, 2.0, 2.3, 2.7 and 4.0 nm, respectively, were prepared from kanemite (layered polysilicate) using alkyltrimethylammonium $[C_nH_{2n+1}N^+(CH_3)_3]$ with different alkyl-chain lengths (n = 10, 12, 14, 16 and 22), according to the method reported by Inagaki *et al.*[6] The BET surface area and pore size analyzed, determined by the BJH method with a nitrogen adsorption isotherm, are listed in Table 1. An FSM was suspended in a 0.1% NaOH ethanol solution for 3 min to obtain Na-FSM. The resulting product was washed with ethanol followed by filtration and air-drying at 50 °C.[9]

Preparation of chlorophyll–FSM conjugates

To 2.0 ml of a benzene solution of chlorophyll *a* (0–32.5 mM) was added 100 mg of Na-FSM-10, -12, -14, -16 or -22. The suspension was then shaken for 30 min at 25 °C to establish the adsorption equilibrium. The chlorophyll–FSM conjugate was collected by centrifugation and then dried under reduced pressure at 25 °C. The amount of chlorophyll *a* adsorbed in the pores of the FSM was spectrophotometrically determined by measuring the absorbance of the supernatant obtained on centrifugation at 665 nm, which is the characteristic absorption of chlorophyll *a*. The molar extinction coefficient of chlorophyll in benzene was determined to be $\varepsilon_{665\ nm} = 9.0 \times 10^4\ M^{-1}\ cm^{-1}$, using that of chlorophyll in acetone, $\varepsilon_{661.6\ nm} = 9.25 \times 10^4\ M^{-1}\ cm^{-1}$.[10] The chlorophyll–FSM conjugate was collected by centrifugation and then dried under reduced pressure, followed by suspension in water. The

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Table 1 Physicochemical and spectrophotometric properties of chlorophyll–FSM conjugates

	Characteristics of host material		Properties of chlorophyll–FSM		
Host material	BJH pore diameter/nm	Specific surface area/m^2 g^{-1}[a]	Chlorophyll *a* adsorbed/mg (100 mg of FSM)$^{-1}$	Absorption maximum/nm	Stabilization of chlorophyll *a* (%)[b]
FSM-22	4.0	1040	29.2	677	100
FSM-16	2.7	1031	24.6	675	97
FSM-14	2.3	1017	15.8	673	52
FSM-12	2.0	923	4.0	671	—
FSM-10	1.6	999	0.75	670	50
Silica gel	—	650	0.70	671	—
Chlorophyll *a* in benzene				665	<8
Chlorophylls in intact leaves				678	100

[a]Before the 0.1% NaOH treatment. [b]Relative absorbance value at the absorption maximum in the red region after 360 min illumination. The absorbance value of each sample obtained before irradiation was regarded as 100%.

amount of conjugate in the suspension was determined and the molar extinction coefficient of chlorophyll–FSM in a 0.1 M tris-HCl buffer (pH 7.4) was determined to be $\varepsilon_{\text{at 675 nm}} = 5.0 \times 10^4$ M^{-1} cm^{-1}.[9]

The absorption spectrum of a chlorophyll–FSM conjugate in water was measured with a Shimadzu spectrophotometer MPS-2400 (Kyoto, Japan).[11]

Photostability of the chlorophyll–FSM conjugate

The photostability of chlorophyll *a* was examined by illuminating a chlorophyll–FSM conjugate in water, as well as that of free chlorophyll *a* in benzene, with a 200 W xenon lamp, the light intensity on the sample being approximately 380 J m^{-2} s^{-1}. Ultraviolet rays of wavelengths shorter than 390 nm were cut out with a Toshiba L-39 filter (Tokyo, Japan). During the illumination, the sample was shielded from heat by means of circulation of cold water.

Results and discussion

Adsorption isotherm

The adsorbed amounts of chlorophyll *a* for FSM-10, -12, -14, -16 and -22 powder in benzene were spectrophotometrically measured with respect to the equilibrium concentration of chlorophyll *a* (Fig. 1). The adsorption of chlorophyll in the pores of FSMs of pore diameter 1.6, 2.0, 2.3, 2.7 and 4.0 nm are shown in B, C, D, E and F, respectively. The amount of chlorophyll adsorbed in the pores of FSMs increased with the FSM pore diameter. The adsorption proceeded efficiently with the equilibrium concentration of chlorophyll *a*, and reached a constant level with an adsorption equilibrium at 6 mM (curve F). In this case, 29 mg of 57 mg of chlorophyll *a* was adsorbed in the pores of FSM (100 mg) in benzene. On the other hand, the amounts of chlorophyll *a* adsorbed on silica gel, FSM-10 and FSM-12 were low in comparison with that in the case of FSM (curves A, B and C). Fig. 2 is a plot of the pore diameter of FSM against the amount of chlorophyll adsorbed in the pores of FSM. Although FSMs with different pore diameters have almost the same surface area, the amount of chlorophyll adsorbed by FSM steeply increases with the pore diameter of 2 nm. This indicates that chlorophyll *a* is adsorbed in the pores of FSM with a pore diameter of 2 nm.

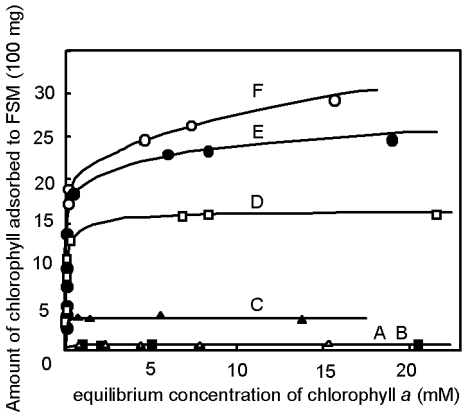

Fig. 1 Adsorption of chlorophyll in the pores of FSMs with 1.6, 2.0, 2.3, 2.7 and 4.0 nm pores (B, C, D, E and F, respectively), and silica gel (A) in benzene was spectrophotometrically measured with respect to the equilibrium concentration of chlorophyll *a*.

Photostability of chlorophyll–FSM conjugates

The absorption maxima of the conjugates are listed in Table 1. The maximum for chlorophyll *a* in benzene was 665 nm, which shifted to a longer wavelength of 670–677 nm for the conjugates with FSM.

Although absorption at 673 nm was also observed for the chlorophyll–smectite,[3] absorption at 677 nm, which is close to that for intact leaves, was observed only for the conjugates of FSM with larger pores. The photostability data on illumination in visible light are shown in Fig. 3. Free chlorophyll *a* in benzene, with an absorption maximum at 665 nm, was markedly discolored by illumination, as is shown by curve A. On the other hand, the chlorophyll–FSM conjugates in water were much more photostable in comparison with free chlorophyll. The photostabilization values of the chlorophyll conjugates with pore diameters of 1.6, 2.3, 2.7 and 4.0 nm are shown by curves B, C, D and E, respectively. The photostability of chlorophyll *a* in FSM pores increased with the pore

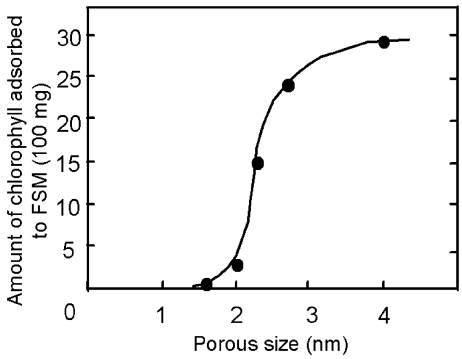

Fig. 2 Relationship between the amount of chlorophyll adsorbed in the pores of FSM (100 mg) and the pore diameter of FSM in the chlorophyll–FSM conjugates.

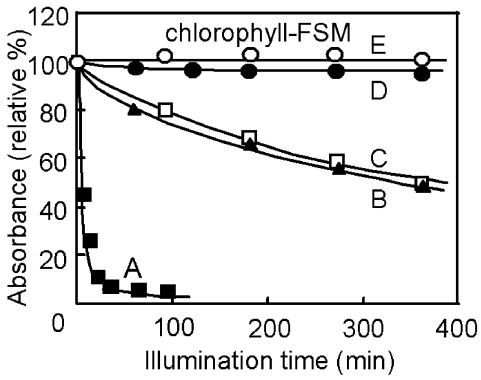

Fig. 3 Photostability of chlorophyll *a* (Chl) and Chl–FSM. Curve A: chlorophyll *a* in benzene. Curves B–E: Chl–FSM conjugates dispersed in water with pore diameters of 1.6, 2.3, 2.7 and 4.0 nm, respectively. Each sample was illuminated at a light intensity of 380 J m^{-2} s^{-1} at 30 °C.

size. A relative absorbance value of 100% is retained at the absorption maximum in the red region after 360 min illumination of curve E. The chlorophyll *a* in FSM-14, whose pore size just fits the guest molecules (Fig. 4), shows insufficient photostability compared with that in FSM-16 and -22. These results suggested that enhancement of the photostability of chlorophyll in conjugates is accompanied not only by the interaction between chlorophyll and the support, but also by an interaction between chlorophyll molecules. In Table 1, the photostabilites are also listed. It is noteworthy that an increase in the pore diameter of FSM is accompanied by a shift of the absorption maximum to a longer wavelength, and also by enhancement of the photostability of the chlorophyll–FSM conjugate. Such a shift of the absorption band is attributable to the interaction between the two chlorophyll molecules in an arrangement shown in Fig. 4.[12] The arrangement of the chlorophyll molecules resembling that in natural leaves is realized in the nano-spaces in FSM, which would play an important role as to the excellent photostability.

Conclusions

Generally, chlorophyll derivatives are not photostable under aerobic conditions.[4,13] However, chlorophyll–FSM conjugates are extensively stable in an aqueous solution in comparison with chlorophyll. Furthermore, chlorophyll *a* is adsorbed in the pores of FSM-16 and -22, having absorption maxima of 675 and 677 nm, respectively. Chlorophylls in intact leaves exhibit an absorption maximum at 678 nm and are quite stable against light. A conceivable reason for this phenomenon may be not only an interaction of the tetrapyrrole ring carrying magnesium

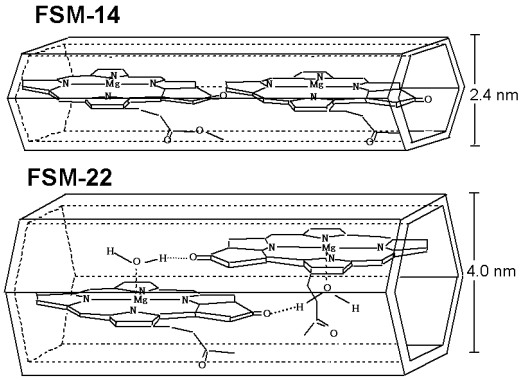

Fig. 4 Tentative arrangement in chlorophyll–FSM.

with the surfaces of the pores of FSM but also an interaction between two chlorophyll molecules yielding a chlorophyll dimer (Fig. 4). The pore size of FSM-22, 4.0 nm, should be suitable for such a dimer-like arrangement. Chlorophyll molecules conjugated with mesoporous compounds could be designed to allow artificial photosynthetic reactions such as water splitting and carbon dioxide fixation.

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