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Photostabilization of Chlorophyll *a* Adsorbed onto Smectite

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ABSTRACT

Chlorophyll a *was adsorbed onto a synthetic smectite, hectorite, in benzene to form a chlorophyll–smectite conjugate. The adsorption proceeded in two steps, involving monolayer and then multilayer adsorption of chlorophyll* a *onto smectite. The chlorophyll–smectite conjugate became a transparent colloidal solution with a green color on the addition of water. The absorption maximum of chlorophyll* a *in the red region shifted to longer wavelength by increasing the amount of chlorophyll* a *adsorbed onto smectite, accompanied by an effective photostabilization.*

INTRODUCTION

It is well known that chlorophyll *a* plays an important role in the primary step of photosynthesis, i.e. the photolysis of water. Chlorophyll molecules bind to proteins to form chlorophyll–protein conjugates in the chloroplast thylakoid membrane *in vivo*, and exhibit physiological functions.[1] With the objective of making an artificial model of the chlorophyll–protein conjugate in green plants, chlorophylls have been mixed with synthetic macromolecules such as polyethylene glycol, polyvinylpyrrolidone and polyvinyl alcohol.[2-4]

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Recently, chlorophyll *a* was adsorbed on to freeze-dried bentonite in benzene to form a chlorophyll–bentonite conjugate. The conjugate was found to be photostable, and the absorption maximum of the conjugate was located at 678 nm, which is in good agreement with that of intact spinach leaves.[5] Trioctahedral smectite (hectorite) was synthesized hydrothermally from homogeneous Si–Mg hydrous oxide. Surprisingly, the dispersed solution of the smectite in water was a transparent gel with attractive properties, i.e. cation exchange, swelling in water and forming organic and inorganic interlayer complexes.[6]

The present paper deals with the preparation of chlorophyll *a* adsorbed onto smectite, and also with enhanced properties of the resultant chlorophyll–smectite conjugate.

EXPERIMENTAL

Chlorophyll *a* from *Spirulina* was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Synthetic smectite powder (hectorite) was obtained from Co-op Chemical Co., Ltd (Tokyo, Japan). The properties of the trioctahedral smectite powder were as follows: elemental composition Si 8·00, Mg 5·65, Li 0·70, Na 1·05; transmittance 95% in 1% aqueous solution at 500 nm; Methylene Blue adsorption 101 meq per 100 g.

The chlorophyll–smectite conjugate was prepared as follows. Smectite powder (300 mg) was added to 1·0 ml of chlorophyll *a* dissolved in benzene (0–41·1 mM). The suspension was then shaken for 1 h at 25°C to establish the adsorption equilibrium between chlorophyll *a* and smectite. The amount of chlorophyll *a* adsorbed onto smectite was spectrophotometrically determined by measuring the absorbance of the supernatant liquor of the sample suspension after centrifuging. The molar extinction coefficient of chlorophyll *a* was $9{\cdot}25 \times 10^4$ M^{-1} cm^{-1} at 661·6 nm in acetone.[7] The chlorophyll–smectite conjugate was collected by centrifugation and was dried under reduced pressure, followed by dissolution in water.

The absorption spectrum of the chlorophyll–smectite conjugate in water, a transparent colloidal solution with green color, was measured on a Shimadzu spectrophotometer MPS-2000 (Kyoto, Japan). The photostability of chlorophyll *a* was tested by irradiating the chlorophyll–smectite conjugate in water, as well as free chlorophyll *a* in benzene, with a 60 W incandescent lamp at a distance of 10 cm; fluence rate of the irradiation on the sample was approximately 2100 W m^{-2}. During the irradiation, the sample was shielded from irradiation heat by the circulation of cold water.

RESULTS AND DISCUSSION

The chlorophyll–smectite conjugates, in which various amounts of chlorophyll *a* (0–0·34 mg) were adsorbed onto 100 mg of smectite, were dissolved in water to form a green colored transparent solution. Absorption spectra of the conjugates (0·5% in water) are shown in Fig. 1(a). Although free chlorophyll *a* in benzene has an absorption maximum at 665 nm, the chlorophyll–smectite conjugates shift to a longer wavelength, i.e. 669 nm (curves A–D). The absorption spectrum of smectite solution without chlorophyll *a* is shown by curve E. The absorbance of smectite without chlorophyll *a* is negligible in the visible region. The absorbance of chlorophyll *a* at 669 nm increases linearly as the amount of chlorophyll *a* adsorbed onto the smectite is increased (Fig. 1(b)). Curves A and B show the absorbance of 1·0% and 0·5% solutions of chlorophyll–smectite conjugates in water. Each linear plot intersects at the zero point in Fig. 1(b), indicating the green colored transparent colloidal solution of the conjugate in water.

The adsorption of chlorophyll *a* onto smectite powder in benzene was spectrophotometrically measured with respect to time (insert in Fig. 2). The adsorption proceeds efficiently with time, and reaches a constant level with adsorption equilibrium within 1 h. In this case, 0·51 mg of the 0·78 mg of chlorophyll *a* is adsorbed onto 100 mg of smectite in

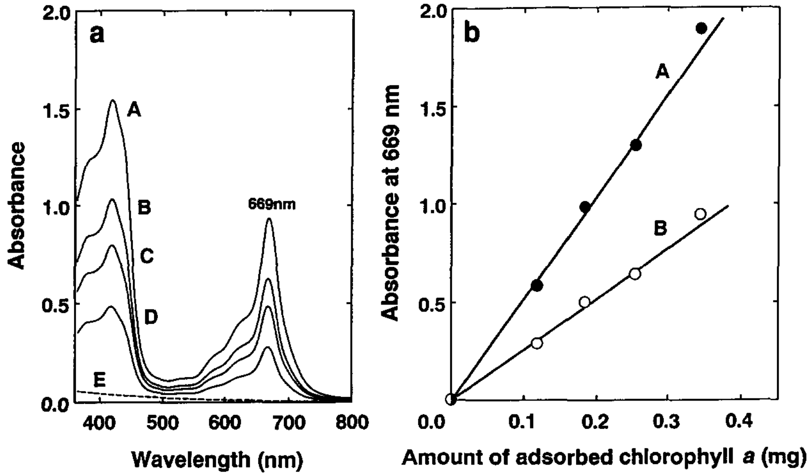

Fig. 1. (a) Absorption spectra of chlorophyll–smectite conjugates (0·5% in water). Curves show amount of chlorophyll *a* adsorbed onto 100 mg of smectite: A, 0·34; B, 0·26; C, 0·18; D, 0·12; E, 0 mg. (b) Plot of the absorbance of chlorophyll–smectite conjugates in water at 669 nm against the amount of adsorbed chlorophyll *a*. Line A 1·0% and line B 0·5% solution of chlorophyll–smectite conjugates in water.

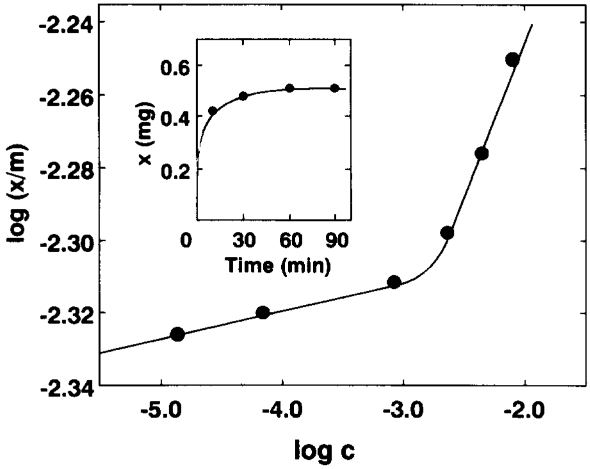

Fig. 2. Adsorption isotherm of chlorophyll *a* onto smectite at 25°C according to the Freundlich equation. Insert shows time-course of the adsorption of chlorophyll *a* onto smectite.

benzene. The adsorption of chlorophyll *a* onto smectite was analysed according to the Freundlich equation:

$$\log (x/m) = \log k + 1/n \log c$$

in which x, m and c represent the amount of chlorophyll *a* adsorbed on to smectite, the amount of adsorbent (100 mg), and the equilibrium concentration of chlorophyll *a*, respectively, and k and n are constants. The adsorption isotherm of chlorophyll *a* is shown in Fig. 2, in which the adsorption proceeds in two steps with a significant change at one point. The adsorption coefficient ($1/n$) was $7{\cdot}6 \times 10^{-3}$ for the first step and $8{\cdot}7 \times 10^{-2}$ for the second step. These results indicate that chlorophyll *a* molecules may bind to the surface of smectite particles as a monolayer (the first step), and then may be stacked on the surface of smectite to make a multilayer of chlorophyll *a* (the second step).

During the course of the adsorption of chlorophyll *a* onto smectite, it was found that the absorption maximum of chlorophyll *a* was shifted to a longer wavelength. Figure 3 is a plot of the wavelength of the absorption maximum against the amount of chlorophyll *a* adsorbed onto the smectite. The peak position shifts to a longer wavelength as the amount of chlorophyll *a* adsorbed increases. The conjugate composed of 1·03 mg of chlorophyll *a* and 100 mg of smectite shows an absorption maximum at 673 nm, while free chlorophyll *a* in benzene has an absorption maximum at 665 nm (Table 1).

Chlorophyll *a* free from protein is quite unstable and is rapidly bleached by light. Figure 4 shows the photostability on irradiation with visible light of chlorophyll–smectite conjugates, together with that of

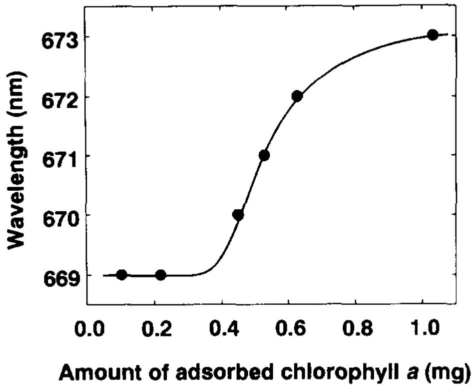

Fig. 3. Relation between the wavelength of the absorption maximum of chlorophyll *a* adsorbed onto smectite and the amount of chlorophyll *a* adsorbed onto smectite (100 mg).

chlorophyll *a* free from smectite. Free chlorophyll *a* in benzene, with an absorption maximum at 665 nm, is markedly discoloured by the irradiation, as is shown by curve A. On the other hand, the chlorophyll–smectite conjugates are much more photostable. The photostabilization of the chlorophyll–smectite conjugates with absorption maxima at 669, 670, 671, 672 and 673 nm are shown by curves B, C, D, E and F, respectively. They appear to become more photostable as the amount of chlorophyll *a* adsorbed is increased. Table 1 shows the properties of the conjugates with different amounts of chlorophyll *a* adsorbed onto smectite, the absorption maximum and the degree of photostability. It is noteworthy that an increase in the amount of chlorophyll *a* adsorbed onto smectite is

TABLE 1
Photostabilization of Chlorophyll *a* Adsorbed onto Smectite

Smectite (mg)	*Chlorophyll adsorbed (mg)*	*Absorption maximum (nm)*	*Stabilization of chlorophyll*[a] *(%)*
100	1·03	673	85·4
100	0·63	672	82·8
100	0·53	671	80·2
100	0·45	670	76·2
100	0·22	669	66·5
Chlorophyll *a* in benzene		665	0

[a] Relative value of absorbance at absorption maximum in the red region after 210 min irradiation. The absorbance value of each sample obtained before the irradiation was regarded as 100%.

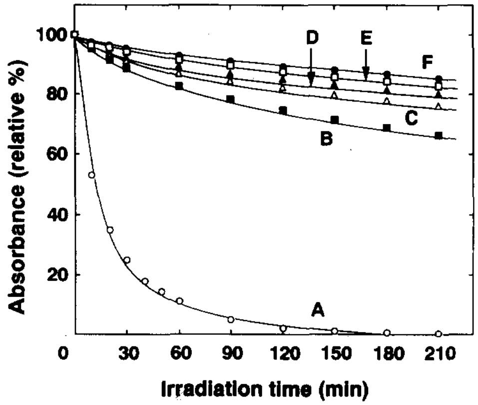

Fig. 4. Photostabilization of chlorophyll–smectite conjugates. Curve A, chlorophyll *a* in benzene; curves B–F, chlorophyll–smectite conjugates dispersed in water with absorption maxima at 669, 670, 671, 672 and 673 nm, respectively. Samples were irradiated with a 60 W incandescent lamp at 10 cm.

accompanied by a shift of the absorption maximum to longer wavelength, and also by an enhancement of the photostability of the chlorophyll–smectite conjugate.

As a result of this investigation, it is suggested that natural pigments which are insoluble in water become soluble by conjugating with smectite; we have successfully prepared a water-soluble xanthophyll–smectite conjugate.

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