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Photostability of biliverdin bound to smectite, clay mineral

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Abstract

Biliverdin, the precursor of bilirubin, was adsorbed onto the synthetic clay mineral, smectite, in a mixed solution of benzene and methyl alcohol (7:3 v/v) to form a biliverdin–smectite conjugate. The adsorption of biliverdin to smectite followed the Freundlich equation with an adsorption coefficient of 1.0. The water-soluble conjugate had a green color with the absorbance maxima at 679 and 378 nm. The biliverdin–smectite conjugate became photostable in water to irradiation with UV, although the biliverdin in benzyl alcohol was rapidly decolorized.

Keywords: Biliverdin; Porphyrin; Synthetic clay mineral; Smectite; Photostability; Bio-conjugate

1. Introduction

Study on bio-conjugate compounds has been extensively developed since the late 1970s; the chemical modification of asparaginase with polyethylene glycol (PEG) leads to the disappearance of binding ability to anti-asparaginase serum [1]. PEG-modified hydrolytic enzyme, lipase, catalyzes effectively the reverse reaction of hydrolysis in hydrophobic media, ester synthesis and ester exchange reactions, in organic solvent [2].

Recently, bio-conjugate materials of the compound porphyrin such as chlorophyll *a* and heme (or hemin) bound to clay mineral, smectite, were reported. The chlorophyll *a*–smectite conjugate became a transparent colloidal solution with a green color in water and was photostable against light irradiation [3]. The conjugate also catalyzed photoreduction and hydrogen gas evolution by visible light [4]. Furthermore, hemin (Fe^{3+})–smectite and heme (Fe^{2+})–smectite conjugates were also soluble in water and their absorption spectra closely agreed with those of oxy- and carboxyhemoglobins, in the presence of O_2 and CO [5]. Although porphyrin pigments have the physiological functions in the living body, the functions are easily lost by the degradation of pigments in vitro. The stabilization of pigments bound to smectite has a possibility of expanding the applications with novel functions of natural pigments.

The chemical structures of biliverdin, unfolded porphyrin ring, and smectite are shown in Scheme 1A and B, respectively. Biliverdin, tetrapyrrole, is a metabolic product from heme with a porphyrin ring and has a green color in organic solvents. In the living body, biliverdin is changed to bilirubin with biliverdin reductase. Biliverdin is degraded with high pH and/or light irradiation [6,7].

In the present study, we have made a bio-conjugate of biliverdin bound to smectite, clay mineral, with

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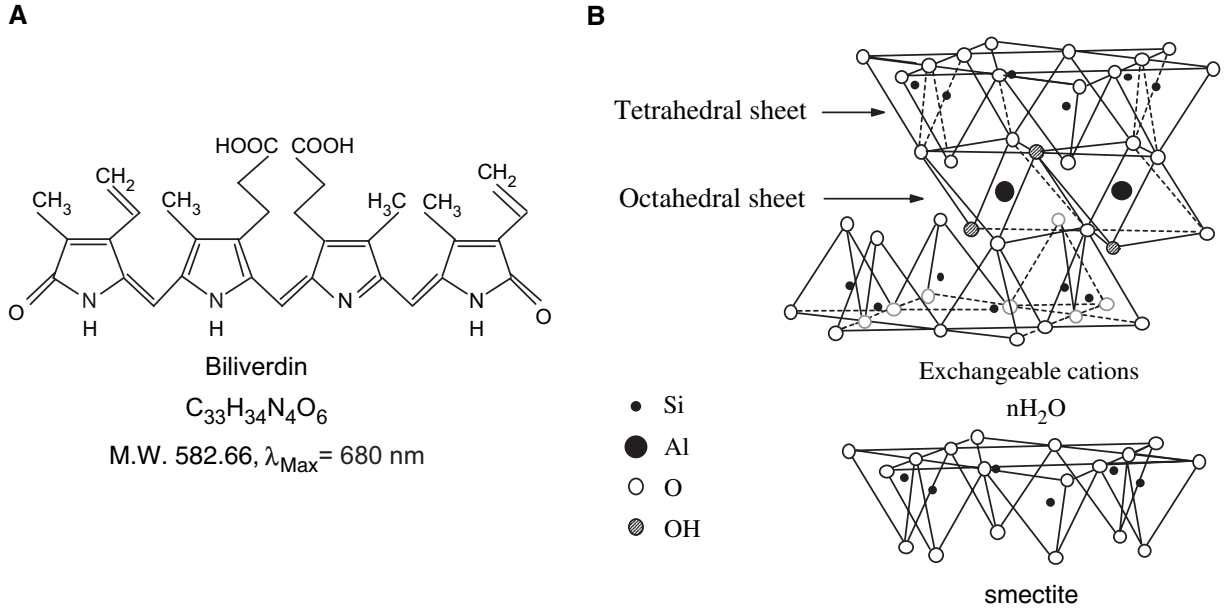

Scheme 1. The chemical structures of biliverdin (A) and smectite (B).

photostability for expanding the availability of natural pigments.

2. Experimental

Biliverdin dihydrochloride, $C_{33}H_{34}N_4O_6{\cdot}2HCl$, was purchased from ICN Biomedicals Inc. (OH, USA). Smectite powder (hectorite), which was hydrothermally synthesized, was obtained from Co-op Chemical Co., Ltd. (Tokyo, Japan). Its properties are as follows: the elemental composition with Si 8.00, Mg 5.65, Li 0.70, Na 1.05; transmittance 95% in 1% aqueous solution at 500 nm; and methylene blue adsorption 101 mequiv per 100 g [8]. The absorption spectra of pigments were measured with Multi-Purpose Spectrophotometer, MPS-2400 (Shimadzu, Kyoto, Japan).

The biliverdin–smectite conjugate was prepared as follows: smectite powder (100 mg) was added to 1.0 ml of biliverdin dissolved in a mixed solution of benzene and methyl alcohol (7:3 v/v) (0–5.5 mg/ml). The suspension was shaken for 1 h at 25 °C to establish the adsorption equilibrium and obtain the biliverdin–smectite conjugate. Then the sample solution was centrifuged and its precipitate dried under reduced pressure. The amount of biliverdin adsorbed onto smectite was spectrophotometrically determined by measuring the absorbance of biliverdin in the supernatant of the sample suspension after centrifuging. The molar extinction coefficient of biliverdin was $2.80 \times 10^4\ M^{-1}\,cm^{-1}$ at 680 nm in methyl alcohol including 5% (w/v) HCl [9].

The absorption spectrum of the biliverdin–smectite conjugate in water, a transparent colloidal solution with green color, was measured by using MPS-2400. Photostability of biliverdin–smectite conjugate was tested as follows: the conjugate in water (2 mg/ml) was irradiated by UV-rays (365 nm, 40 W) at a distance of 16 cm at room temperature. The absorbance change of biliverdin–smectite conjugate at 679 nm was measured against irradiation time. A similar test was carried out with free biliverdin in benzyl alcohol (12 μg/ml) at room temperature by the same UV irradiation at a distance of 16 cm.

3. Results and discussion

The biliverdin–smectite conjugates, in which various amounts of biliverdin (0–5.42 mg) were adsorbed onto 100 mg of smectite, were dissolved in water to form a green colored transparent solution. Absorption spectra of the conjugates (1.0% in water) are shown in Fig. 1a. The absorbance peaks at 679 nm and 378 nm are enhanced by increasing the amount of biliverdin bound to smectite as is shown in curves D, C, B and A. The absorption spectrum of smectite soluble in water (1.0%) without biliverdin is negligible at the visible region from 800 to 300 nm as is shown in curve E.

The adsorption of biliverdin onto smectite powder in a mixed solution of benzene and methyl alcohol (7:3 v/v) was spectrophotometrically measured with respect to time (insert in Fig. 1b). The adsorption proceeds efficiently with time, and reaches a constant level with adsorption equilibrium within 1 h. In this case, 0.60 mg out of 0.63 mg biliverdin is adsorbed onto 100 mg of smectite in a mixed solution of benzene and methyl alcohol (7:3 v/v). The adsorption of biliverdin onto

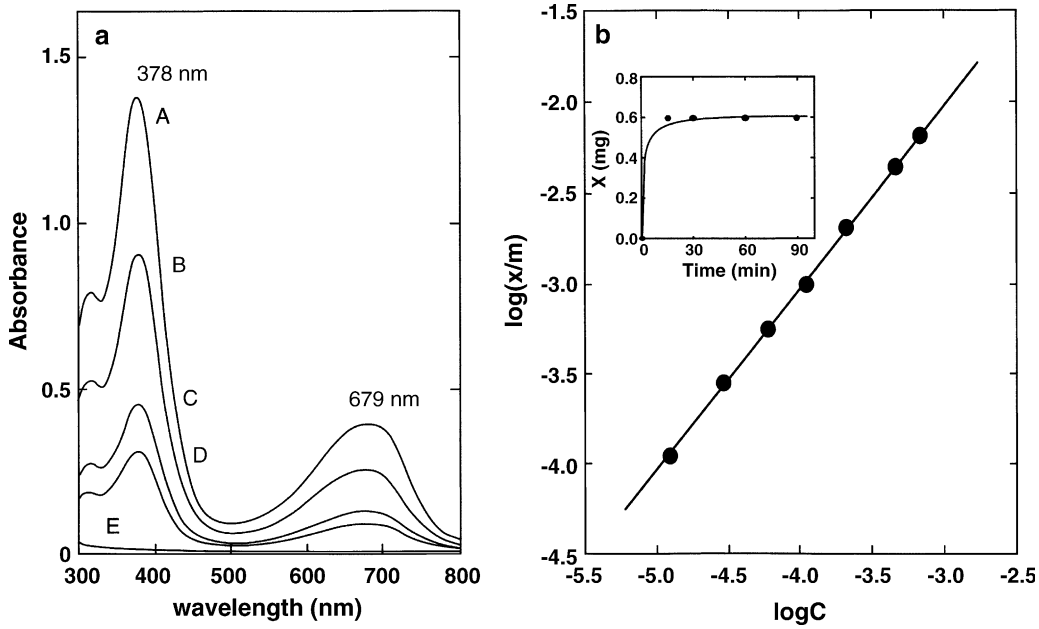

Fig. 1. (a) Adsorption spectra of biliverdin–smectite conjugates in water. Curves A, B, C, D and E: 5.42, 2.24, 0.86, 0.37 and 0 mg of biliverdin adsorbed onto 100 mg of smectite. (b) Adsorption isotherm of biliverdin onto smectite at 25 °C according to the Freundlich equation. Insert shows time-course of the adsorption of biliverdin onto 100 mg of smectite.

smectite was analyzed according to the Freundlich equation:

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

in which x, m and c represent the amount of biliverdin adsorbed onto smectite, the amount of adsorbent (100 mg), and the equilibrium concentration of biliverdin, respectively. k and n are constants. The adsorption isotherm of biliverdin is shown in Fig. 1b. The adsorption coefficient ($1/n$) was 1.0. These results indicated that biliverdin molecule may bind to the surface of smectite particle as mono- and multi-layers.

The next series of experiments is concerned with the photostabilization of biliverdin, bound to smectite, to irradiation of UV-rays. The absorption spectrum of biliverdin has two absorption bands at 671 nm and

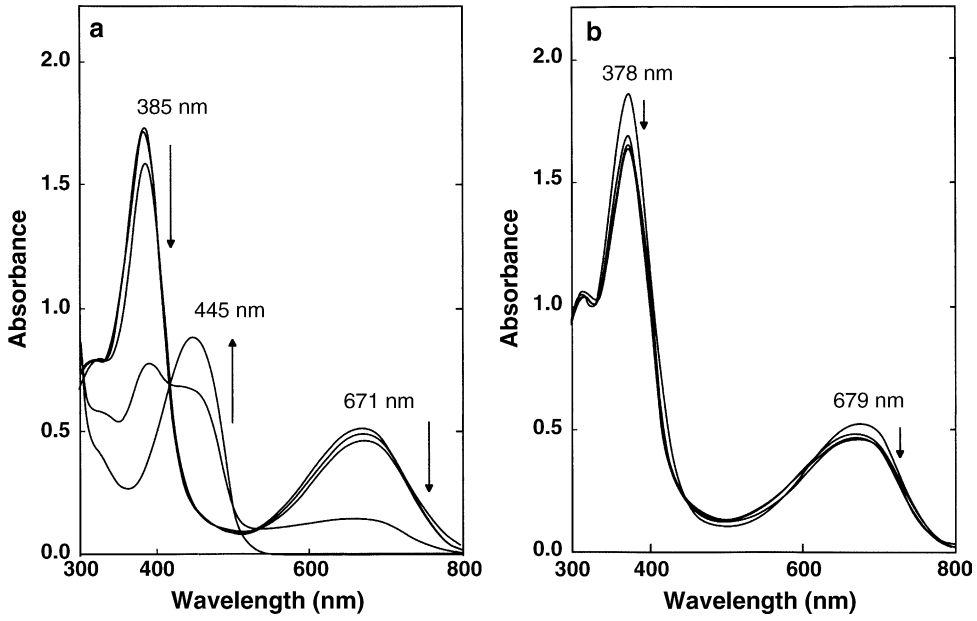

Fig. 2. Spectral changes of biliverdin in benzyl alcohol (a) and of biliverdin–smectite conjugate in aqueous solution (b) under UV irradiation. Each sample was irradiated with 40 W UV-rays (365 nm) from a distance of 16 cm. The absorption spectra were measured after irradiation for 0, 5, 10, 20, 30 and 60 min.

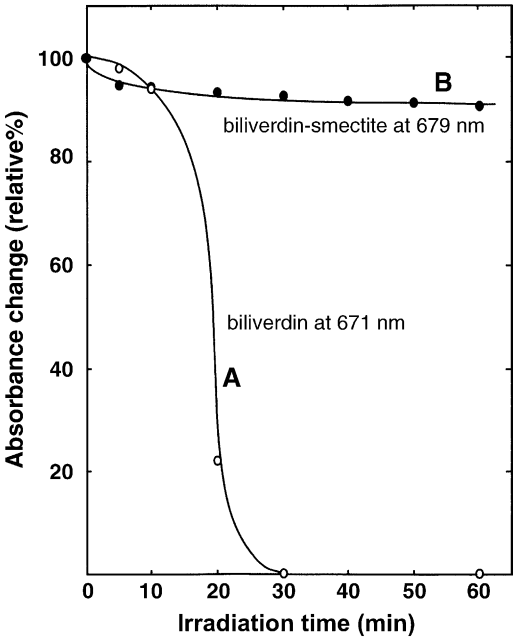

Fig. 3. Photostability of biliverdin–smectite conjugate and biliverdin under irradiation with a 40 W UV-rays (365 nm) from a distance of 16 cm at room temperature. Curve A: absorbance decrease at 671 nm of biliverdin dissolved in benzyl alcohol (12 μg/ml); curve B: absorbance decrease at 679 nm of biliverdin–smectite conjugate dissolved in distilled water (2.0 mg/ml).

385 nm as is shown in Fig. 2a. By irradiation of UV-rays, the absorption bands of two peaks are lowered with time and new absorption band appears at 445 nm. The spectral change has an isobestic point at about 420 nm, indicating the formation of bilirubin with absorption maxima with 445 nm in benzyl alcohol [9]. A similar experiment was carried out for biliverdin–smectite conjugate in aqueous solution exposed to irradiation of UV-rays for 1 h. The two peaks at 679 nm and 378 nm were hardly lowered by UV irradiation as is shown in Fig. 2b. The absorbance changes of biliverdin (at 671 nm) and biliverdin–smectite (at 679 nm) were plotted against irradiation time (0–60 min) (Fig. 3). Although biliverdin was rapidly decolorized by irradiation of UV for 30 min as is shown in curve A, the biliverdin–smectite conjugate maintained approximately 91% of color development even for 1 h under UV irradiation (curve B).

The solubility of biliverdin–smectite conjugate in water depends upon the amount of biliverdin adsorbed onto smectite (Table 1). As is shown in Table 1, amounts of biliverdin (mg) adsorbed onto 10 mg of smectite range from 1.3 to 6.5 mg. Within this range, the conjugates soluble in water are limited to 2.9–1.3 mg of biliverdin onto 10 mg of smectite. Further the solubility in water was reduced with increasing amounts of adsorbed biliverdin.

Table 1
Solubility of biliverdin–smectite conjugate in water

Amount of biliverdin adsorbed onto 10 mg of smectite (mg)	1.3	2.3	2.9	6.3	6.5
Solubility in water	+++	++	+	–	–

Relation between the amounts of biliverdin adsorbed onto 10 mg smectite and their solubility in water.

From the results obtained together with previous reports [3–5] it can be concluded that natural pigments, including chlorophyll *a*, heme (or hemin), and biliverdin become photostable and soluble in water by binding with the clay mineral, smectite.

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