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Photostabilization of phycocyanin and anthocyanin in the presence of biopterin-α-glucoside from *Spirulina platensis* under ultraviolet ray

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Abstract

Biopterin-α-glucoside purified from *Spirulina platensis* is stable to irradiation by visible and ultraviolet light. Its absorption spectrum shows maxima at 268 nm and 340 nm and a fluorescence band appears at 447 nm. Although cyanidin-3-galactoside with an absorption maximum at 514 nm (red colour) is unstable and is decolourized by UV light, the pigment becomes stable in the presence of biopterin-α-glucoside. Similar photostabilization phenomenon was observed for phycocyanin (λ_{max} 614 nm) consisting of phycocyanobilin as chromophore and protein subunits $\alpha_3\beta_3$ in the presence of biopterin-α-glucoside. Although the chromophore of phycocyanin is related to phycocyanobilin and protein conformation, the chromophore can be stabilized to UV light in the presence of biopterin-α-glucoside.

Keywords: Biopterin; Phycocyanin; Cyanidin; Spirulina; Photostability; Pigment

1. Introduction

Cyanobacteria, including *Spirulina platensis*, have been on the earth for 3.5×10^9 (3.5 billion) years [1]; as is well known, the earth was once an inhospitable environment because of strong UV light content. Spirulina cells have been shown to survive under stringent conditions at high pH (about 10), high HCO_3^- concentration and high UV content [2]. Forrest et al. discovered a fluorescent substance in cyanobacterium which was identified as biopterin-α-glucoside [3]. Noguchi et al. [4] isolated the fluorescent substance in *S. platensis* and determined its structure (Scheme 1).

Phycocyanin is the blue coloured component of spirulina and comprises two kinds of protein, α-subunit (20.5 kDa) and β-subunit (23.5 kDa), with a phycocyanobilin molecule as chromophore [5,6]. Kageyama et al. reported that the chromophore in phycocyanin was closely related to protein conformation and was sensitive to UV light [7]. Cyanidin-3-galactoside, an anthocyanin, can be obtained from young apple plant tissue and has a

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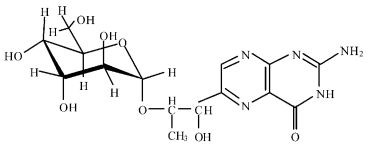


Scheme 1. Structure of biopterin-α-glucoside.

red colour under acidic pH [8]. It is well known that such natural pigments are photosensitive and are bleached by UV light.

The present study concerns the preventing a decolourization of such natural pigments using photostable biopterin-α-glucoside isolated from Spirulina cells.

2. Experimental

Spirulina (*S. platensis*) cells in a dry state were obtained from Spirulina Bio-Lab. Co.

2.1. Isolation of biopterin-α-glucoside, phycocyanin and cyanidin-3-galactoside

Biopterin-α-glucoside was isolated as follows. Dry Spirulina cells (10 g) were extracted three times with 99.8% methyl alcohol (200 ml) to remove chlorophyll. To the residue was added 80% ethyl alcohol (200 ml) to realize biopterin-α-glucoside in the filtrate. The extracted solution was evaporated to remove the alcohol and the resulting dried material was dissolved in 40 ml of water. The solution was subjected to gel chromatography using a Sephadex G-15 (Amersham Biosciences). The ensuing solution was freeze-dried and 20 mg of the biopterin-α-glucoside (purity 20%) obtained.

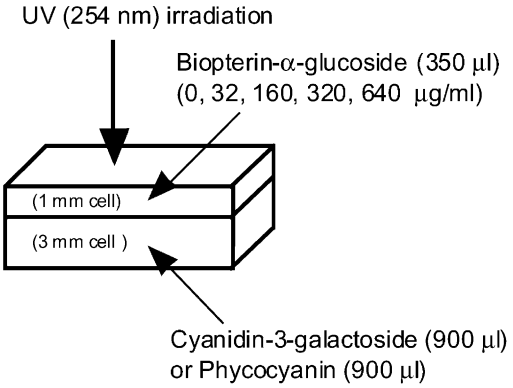
Phycocyanin was isolated from Spirulina (*Spirulina platensis*) cells as previously reported [7]. To 10 g of Spirulina cells were added 400 ml of 50 mM phosphate buffer (pH 7). The ensuing suspension was left for 4 h at 4 °C to extract phycocyanin. The supernatant solution obtained by centrifugation (3000 rpm, 10 min) was added, dropwise, 1.0 M citric acid to adjust the pH 4 (the isoelectric point of phycocyanin). The precipitate was caused by centrifugation at 9000 rpm for 10 min. The crude phycocyanin thus obtained was dissolved in 60 ml of 50 mM phosphate buffer (pH 7), and ion-exchange chromatography (Deae-Toyopearl 650, Tosoh) was used to isolate the phycocyanin. The filtered fraction containing the phycocyanin protein was dialyzed against water and lyophilized.

Anthocyanin (cyanidin-3-galactoside) was purified from young apple cells as follows [8]. To apple cell powder (25 mg) was added 5 ml of methyl alcohol and 1% HCl. After standing for 1 h, the ensuing solution was evaporated to remove methyl alcohol and cyanidin-3-galactoside was obtained as a red paste.

2.2. Photostabilization of pigments towards ultraviolet ray irradiation

Absorption and fluorescent spectra were measured using a Shimadzu MPS-2400 (Kyoto) and a Hitachi Fluorescence Spectrophotometer F-3000 (Tokyo), respectively.

The reaction system consisted of two flat vessels, the upper cell (1 mm light pathway, volume 350 μl) containing biopterin-α-glucoside (0–640 μg/ml) and the lower cell (3 mm light pathway, volume 900 μl) containing pigments, phycocyanin or cyanidin-3-galactoside. UV light (254 nm) was shined onto samples from a height of 17 cm using a UV Crosslinker XL-1000 (40 W) (Spectronics) (Scheme 2). The photostability of the pigments was determined by measuring the absorbance change at the λ_{max} of each pigment (biopterin-α-



Scheme 2. Reaction system of photostabilization.

glucoside: 340 nm, cyanidin-3-galactoside: 514 nm and phycocyanin: 614 nm, respectively).

3. Results and discussion

The absorption spectrum of biopterin-α-glucoside, a fluorescent substance isolated from Spirulina, has absorption maxima at 340 nm and at 268 nm in the ultraviolet region (Fig. 1a). Upon irradiation with UV light, a fluorescence band appeared at 447 nm (Fig. 1b). Photostabilization of biopterin-α-glucoside was determined using UV light (254 nm) irradiation at 25 °C. The results are shown in Fig. 1c, from which it is established that the absorbance at 340 nm was not reduced after 120 min of irradiation. In order to determine the extent of photostability of fluorescence activity, the fluorescence intensity at 447 nm impacted by UV irradiation at 340 nm was measured by increasing the temperature from 25 °C to 70 °C. The results are shown in Fig. 1d (▲), in which the fluorescence intensity decreased with increasing temperature. However, the fluorescence intensity, when reduced at high temperature (70 °C or 40 °C), was reversible and returned to the original fluorescence intensity (△). In contrast, the absorbance of biopterin-α-glucoside at 340 nm remained unaltered when the temperature was changed from 25 °C to 70 °C (●) and from 70 °C to 25 °C (○), as is shown in Fig. 1e.

The chemical structure of cyanidin-3-galactoside (red colour) and its absorption spectrum at pH 1.1 are shown in Fig. 2a. The peak position of the spectrum was located at 514 nm at an absorbance of 0.319 (curve I). When the cyanidin-3-galactoside solution was irradiated with UV light at 254 nm for 120 min, the absorption band disappeared as seen in curve II. To prevent bleaching of cyanidin-3-galactoside, an attempt was made by using biopterin-α-glucoside to determine how decolouration of the pigment could be prevented. Two flat vessels, the upper quartz cell containing biopterin-α-glucoside and the lower quartz cell containing cyanidin-3-galactoside, were irradiated with UV light. In the absence of the fluorescent substance, biopterin-α-glucoside, the absorbance of cyanidin-3-galactoside was reduced to 15%

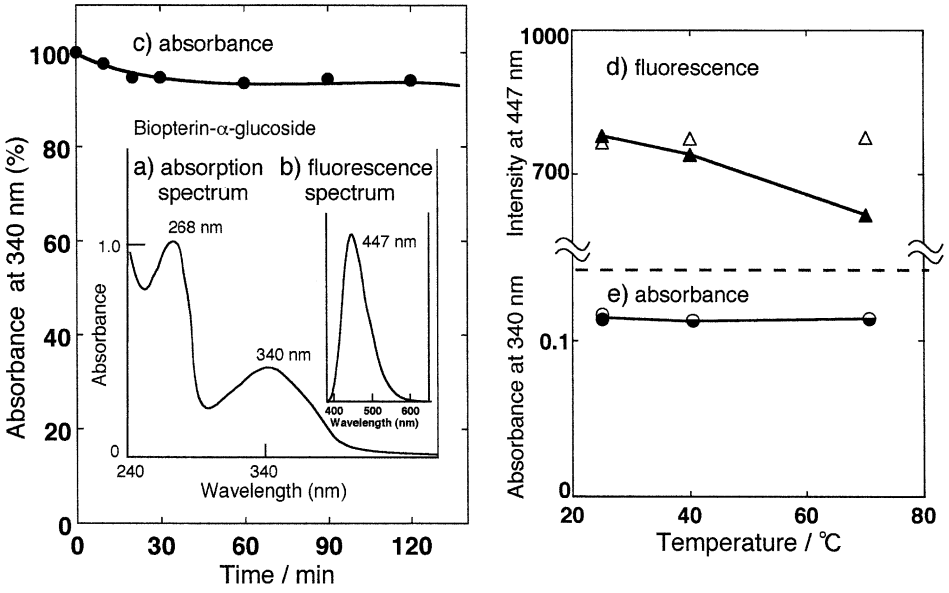


Fig. 1. Photostability of biopterin-α-glucoside against UV irradiation or against high temperatures. (a) and (b) Absorption and fluorescence spectra of biopterin-α-glucoside (26 μg/ml); (c) absorbance change at 340 nm upon UV irradiation; (d) and (e) fluorescence intensity (▲) and absorbance (●) of biopterin-α-glucoside as a function of temperature. Fluorescence intensity (△) and absorbance (○) of biopterin-α-glucoside upon cooling to 25 °C from 70 °C.

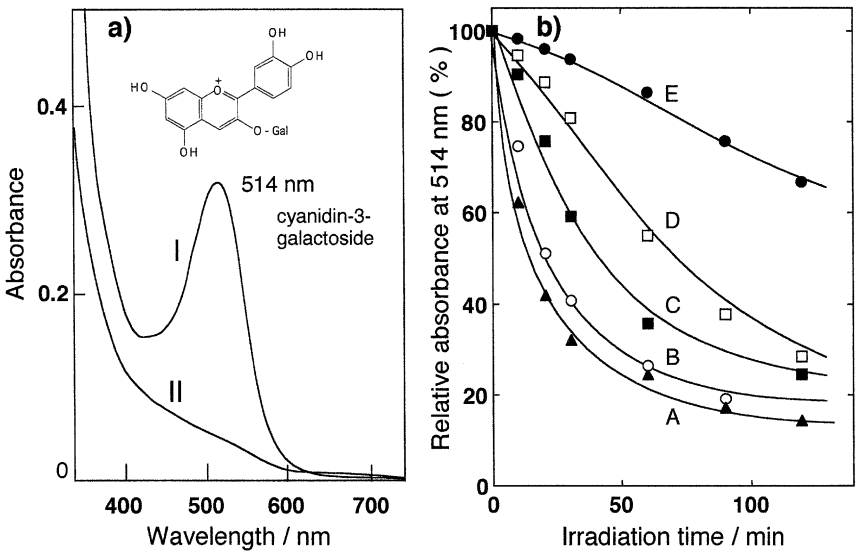


Fig. 2. Photostability of cyanidin-3-galactoside in the presence of biopterin-α-glucoside under UV irradiation. (a) Chemical structure of cyanidin-3-galactoside and its absorption spectrum at pH 1.1 (curve I). Absorption spectrum of cyanidin-3-galactoside photo-xidized by UV light (curve II). (b) Curves A, B, C, D and E corresponding to 0, 32, 160, 320 and 640 μg/ml, respectively, of biopterin-α-glucoside in the upper cell of Scheme 2. The absorbance of cyanidin-3-galactoside in the lower cell at 514 nm was measured after ultraviolet ray irradiation for 0, 10, 20, 30, 60, 90 and 120 min.

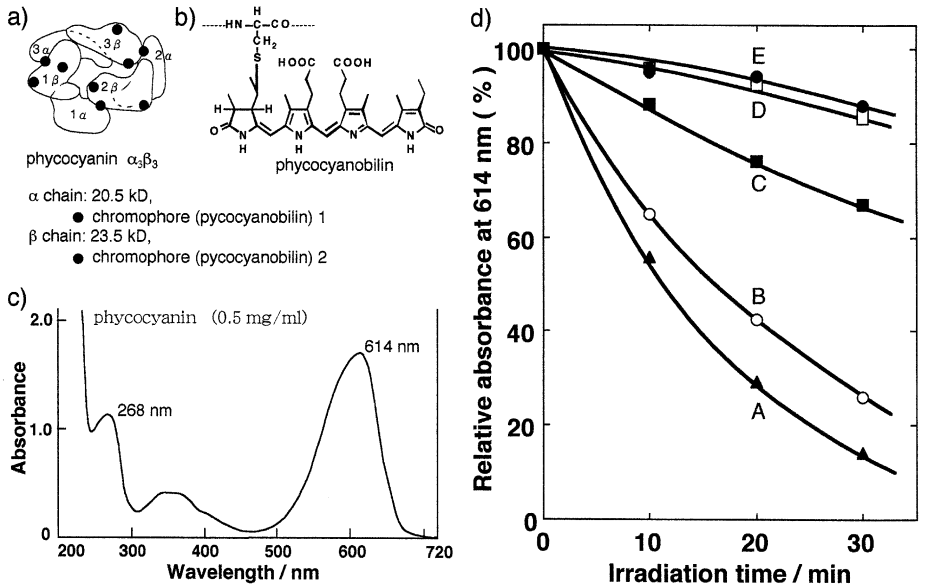


Fig. 3. Photostability of phycocyanin in the presence of biopterin-α-glucoside under UV irradiation at 254 nm. (a) and (b) Conformation of phycocyanin and phycocyanobilin; (c) absorption spectrum of phycocyanin; (d) the two stacked cells, the upper included biopterin-α-glucoside solution and the lower included phycocyanin solution (1.0 mg /ml), were irradiated by ultraviolet ray at 254 nm. Curves A, B, C, D and E corresponding to 0, 32, 160, 320 and 640 μg/ml, respectively, of biopterin-α-glucoside in the upper cell of Scheme 2.

upon UV irradiation for 120 min as seen from curve A in Fig. 2b. By increasing the amount of the fluorescent substance from 0 to 32, 160, 320 and 640 μg/ml, the absorbance of cyanidin-3-galactoside at 514 nm was gradually retained as shown by curves B, C, D and E. From the results obtained, it is seen that decolouration of cyanidin-3-galactoside by UV light was prevented in the presence of biopterin-α-glucoside.

A similar line of study was performed for photostabilization of phycocyanin in the presence and absence of biopterin-α-glucoside under UV light (Fig. 3). Phycocyanin is composed of two kinds of proteins, namely, α-subunit (20.5 kDa) and β-subunit (23.5 kDa) as well as a chromophore, phycocyanobilin [9]. The phycocyanin molecule consists of three sets of α-chain and β-chain, in which phycocyanobilin is present. Phycocyanobilin, one in α-chain and two in β-chain (Fig. 3a and b), is bound to the α- and β-chains by thioether bonds, respectively. The absorption spectrum of phycocyanin is shown in Fig. 3c, in which the absorption maximum is located at 614 nm with blue colour. Fig. 3d shows the photostability of phycocyanin in the presence (curves B–E) and the absence (curve A) of biopterin-α-glucoside under UV light at 254 nm. Although the phycocyanin pigment with an absorption band at 614 nm was rapidly discoloured by UV irradiation (curve A), the pigment was stabilized by increasing the biopterin-α-glucoside concentration (curves B, C, D and E). From the results obtained above, it can be concluded that biopterin-α-glucoside plays an important role in stabilization against UV light.

Natural pigments isolated from plants are generally unstable and decolourized by light irradiation, although beautiful pigments in the plant world are highly appreciated by human beings. To overcome these drawbacks, it was reported that chlorophyll *a*, β-carotene and astaxanthin were adsorbed on the silk protein, fibroin, to form photostable pigment–fibroin conjugates [10]. Furthermore, chlorophyll *a* conjugated with smectite, i.e. chlorophyll–smectite conjugate, became a transparent colloidal solution in water and photostable [11].

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