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Purification, crystallization and preliminary X-ray diffraction analysis of water-soluble chlorophyll-binding protein from *Chenopodium album*

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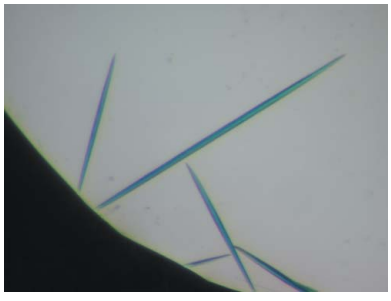

A water-soluble chlorophyll-binding protein (WSCP) with photoconvertibility from *Chenopodium album* was extracted, purified and crystallized in a darkroom. Green crystals suitable for data collection appeared in about 10 d. A native data set was collected to 2.0 Å resolution at 100 K. The space group of the crystal was determined to be orthorhombic $I222$ or $I2_12_12_1$, with unit-cell parameters $a = 48.13$, $b = 60.59$, $c = 107.21$ Å. Preliminary analysis of the X-ray data indicated that there is one molecule per asymmetric unit.

1. Introduction

Generally, chlorophyll (Chl) molecules functioning in photosynthesis are associated with hydrophobic integral membrane proteins. Such Chl molecules are maintained in a functionally effective structure which affords a highly controlled system of energy transfer and charge separation. A water-soluble Chl-binding protein (WSCP) was first found in *Chenopodium album* in 1963 (Yakushiji *et al.*, 1963). WSCPs have so far been detected in several species classified in the Polygonaceae, Chenopodiaceae, Amaranthaceae and Brassicaceae families (Satoh *et al.*, 2001). These WSCPs can be categorized into two classes according to their photoconvertibility: *Chenopodium*-type (class I) and *Brassica*-type (class II). The absorption spectrum of a class I WSCP changes drastically on exposure to visible light, while that of a class II WSCP does not. The amino-acid sequences of class II WSCPs show no similarity to those of class I WSCPs. The *Lepidium virginicum* WSCP, which belongs to class II, consists of four subunits and a Chl molecule is contained in each subunit (Horigome *et al.*, 2007). The physiological function of class II WSCPs has been supposed to be as a Chl-carrier. In contrast, there are no reports of the structure of a class I WSCP and the physiological function of this class of WSCPs has not yet been clarified. In order to determine the crystal structure of a class I WSCP and elucidate the photoconversion mechanism, *C. album* WSCP was extracted, purified and crystallized.

2. Experimental procedures and results

2.1. Purification of *C. album* WSCP

Unless noted otherwise, 25 m*M* sodium dibasic/potassium monobasic phosphate buffer pH 7.0 was used in all purification procedures. *C. album* leaves were harvested in Chiba, Japan. The leaves were washed and homogenized with the buffer and filtered. Solid ammonium sulfate was added to the filtrate and a fraction between 30% and 70% saturation was collected by centrifugation (8000*g*) for 50 min at 277 K. The precipitate was dissolved and dialyzed against the buffer. After the removal of insoluble precipitates by centrifugation (8000*g*) for 30 min at 277 K, the supernatant was applied onto a CM-Cellulofine (Seikagaku Kogyo, Tokyo) column equilibrated with the buffer. The bound protein was washed with the buffer and eluted with 500 m*M* sodium dibasic/potassium monobasic phosphate buffer pH 7.0. The major band was collected and loaded onto a Sephacryl S-200HR (Pharmacia Biotech. Inc.) column equilibrated and eluted with 150 m*M* NaCl and 100 m*M* sodium dibasic/potassium monobasic phosphate buffer pH 7.0. Selected protein fractions were subjected to cation-exchange chromatography on a Resource S (Pharmacia

Biotech. Inc.) column equilibrated with the buffer. The bound protein was eluted with a linear gradient of 25–500 m*M* sodium dibasic/potassium monobasic phosphate buffer pH 7.0. The purified protein was finally subjected to gel-filtration chromatography on a Superdex 200pg (Pharmacia Biotech. Inc.) column equilibrated and eluted with 150 m*M* NaCl and 100 m*M* sodium dibasic/potassium monobasic phosphate buffer pH 7.0. Proteins were separated by 12.5% SDS–PAGE and the proteins on the gel were stained with Coomassie Blue R-250. The protein sample was concentrated using an Amicon concentrator (Millipore).

2.2. Absorption spectra of *C. album* WSCP

The spectrum of purified *C. album* WSCP is shown in Fig. 1. The absorption maxima at 430 and 668 nm diminished in intensity on exposure to visible light, while new peaks appeared at 570 and 740 nm. These spectral changes imply that the Chl molecules have changed their structure.

2.3. Crystallization

Purified *C. album* WSCP was concentrated to 5 mg ml^{-1} in 25 m*M* sodium dibasic/potassium monobasic phosphate buffer pH 7.0. Crystallization conditions were initially screened by means of the sitting-drop vapour-diffusion method at 293 K using crystallization screening kits from Hampton Research. 1 µl *C. album* WSCP solution was mixed with an equal volume of reservoir solution and was equilibrated against 100 µl reservoir solution. The crystals were grown using solution No. 14 from PEG/Ion Screen [200 m*M* potassium thiocyanate (KSCN), 20% polyethylene glycol (PEG) 3350]. The crystallization condition was optimized by screening different types of PEG at different concentrations. The crystallization drops were prepared by mixing 2 µl protein solution and an equal volume of reservoir solution and were equilibrated against 500 µl reservoir solution. The crystals used for data collection were obtained using a reservoir solution containing 3.0 *M* KSCN, 26% PEG monomethyl ether 2000 (Fig. 2). All the experiments were performed in a dark-room; only dim red light was used when necessary.

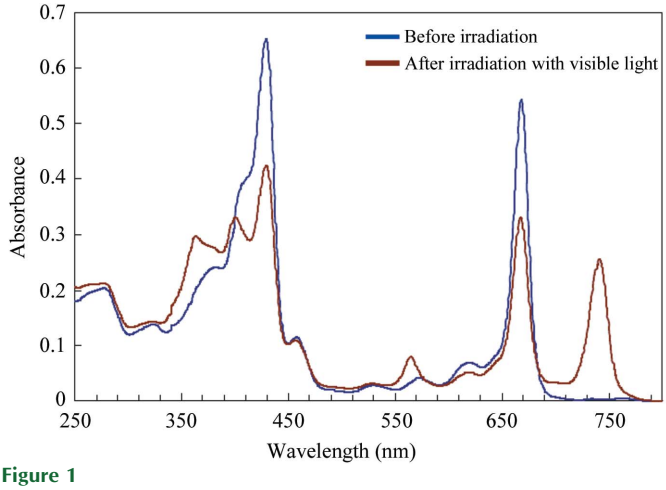

Figure 1
Absorption spectra of *C. album* WSCP before (blue) and after (red) irradiation.

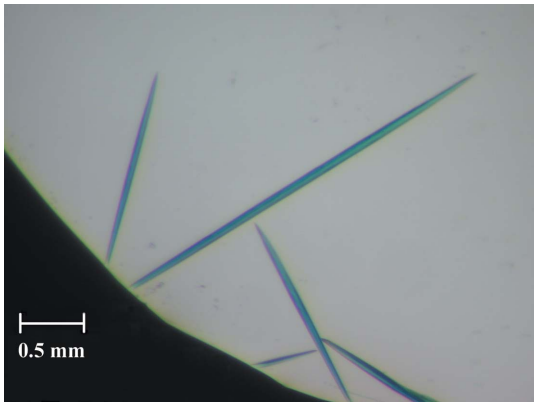

Figure 2
Crystals of *C. album* WSCP.

Table 1
Crystal data and data-collection statistics.

X-ray source	PF BL-5A
Wavelength used (Å)	1.000
Space group	*I*222 or $I2_12_12_1$
Unit-cell parameters	
a (Å)	48.13
b (Å)	60.59
c (Å)	107.21
Unit-cell volume (Å^3)	312599
Resolution range (Å)	43.91–2.02 (2.09–2.02)
Observed reflections	73136
Unique reflections	10628
Completeness (%)	99.99 (100.0)
$R_{\rm merge}$† (%)	7.8 (30.9)
$\langle I/\sigma(I)\rangle$	11.2 (4.8)
Matthews coefficient (Å^3 Da^{-1})	2.4
Solvent content (%)	49.2

† $R_{\rm merge} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i \langle I(h)\rangle$, where $I(h, i)$ is the intensity of reflection h in the ith measurement and $\langle I(h)\rangle$ is the mean value of the intensity of reflection h.

2.4. Data collection and processing

Diffraction data were collected at a wavelength of 1.000 Å using a Quantum 315 CCD detector (ADSC) at the BL-5A station of the Photon Factory (Tsukuba, Japan). The crystals were transferred into a cryoprotectant solution consisting of 3.0 *M* KSCN, 26% PEG monomethyl ether 2000 and 20%(*v*/*v*) glycerol and kept for 1 d; they were then flash-cooled in a stream of nitrogen gas maintained at 100 K. Individual 1° oscillation frames were collected over a range of 180°. All diffraction images were indexed, integrated and scaled using the *CrystalClear* software (Rigaku/MSC) and the scaled data were processed using the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). Statistics of the data collection are given in Table 1. The crystals belong to the orthorhombic space group *I*222 or $I2_12_12_1$, with unit-cell parameters a = 48.13, b = 60.59, c = 107.21 Å. Assuming the presence of one monomer per asymmetric unit, the Matthews coefficient $V_{\rm M}$ is calculated to be 2.4 Å^3 Da^{-1}, which corresponds to a solvent content of 49.2% (Matthews, 1968). An extensive search for heavy-atom derivatives is in progress in order to solve the crystal structure of *C. album* WSCP by SAD or MAD phasing.

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