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CRYSTALLIZATION OF WATER-SOLUBLE CHLOROPHYLL-PROTEINS FROM *LEPIDIUM VIRGINICUM*

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Summary

Water-soluble chlorophyll-proteins were prepared from leaves of *Lepidium virginicum*, by means of ammonium sulfate fractionation followed by column chromatography on DEAE-cellulose and Sephacryl S-200. After intensive purification the chlorophyll-proteins were crystallized by dialysis against an ammonium sulfate solution.

The water-soluble bacteriochlorophyll-protein isolated from the green photosynthetic bacteria by Olson and Romano [1] contains only bacteriochlorophyll *a* and amino acids but no carotenoids [2], and is crystallizable [3]. The X-ray diffraction analysis of the crystals has revealed that seven molecules of bacteriochlorophyll *a* form the central core in the protein subunits [4].

The water-soluble chlorophyll-protein of higher plants was first prepared from *Chenopodium album* by Yakushiji et al. [5]. We also prepared two other types of chlorophyll-protein, one from inflorescence of cauliflower [6] and leaves of *Brassica nigra* [7], and the other from leaves of *Lepidium virginicum* L. All of the proteins contain chlorophyll *a* and *b* but no carotenoids, indicating similarity to the crystallizable bacteriochlorophyll-protein. None of the chlorophyll-proteins from higher plants, however, has been crystallized. In the present paper we will describe the crystallization of chlorophyll-protein from *L. virginicum*.

Leaves of *L. virginicum* were collected in the campus of Toho University at Narashino, Chiba, Japan. Leaves were also collected from the plant which was cultivated in a green-house from seeds harvested near Mono Lake, CA.

Leaves (350 g) were homogenized with a Waring blender in 0.1 M sodium phosphate/potassium phosphate buffer, pH 7.2 (600 ml). The homogenate was filtered through three layers of cheese-cloth. The green filtrate was subjected to $(NH_4)_2SO_4$ fractionation, a fraction precipitating between 40 and 90% saturation being collected. The fraction was dissolved in 0.02 M sodium phosphate/potassium phosphate buffer, pH 7.2 (100 ml). After dialysis against the same buffer solution (5 l), the protein solution was applied to the DEAE-cellulose column (2 cm diameter × 7 cm high). The chlorophyll-protein was adsorbed on the column underneath a brown layer at the top. After the column was washed with 0.02 M sodium phosphate/potassium phosphate buffer, pH 7.2 (250 ml), the chlorophyll-protein was eluted with 0.2 M sodium phosphate/potassium phosphate buffer, pH 7.2. The green solution thus obtained was subjected to a second $(NH_4)_2SO_4$ fractionation, a fraction precipitating between 60 and 90% saturation being collected. The fraction obtained was dissolved in 0.1 M sodium phosphate/potassium phosphate buffer, pH 7.2 (2 ml), and after the insoluble fraction was removed by centrifugation the protein solution was applied to the column of Sephacryl S-200 (2.5 cm diameter × 90 cm high). Fractions with green color (30 ml) were collected, and made 90% saturated in $(NH_4)_2SO_4$. The precipitate was collected and dissolved in 0.1 M sodium phosphate/potassium phosphate buffer, pH 7.2 (1 ml), and dialyzed at 5°C for 2—3 days against 300 ml of 90% saturated $(NH_4)_2SO_4$ solution containing 0.01 M sodium phosphate/potassium phosphate buffer, pH 7.2. Then, crystals of the chlorophyll-protein, dark-green in color, began to appear. After the crystals grew for 20 days, they were collected by centrifugation. Amorphous substances were removed by repeated centrifugation and resuspension of the crystals in 90% satd. $(NH_4)_2SO_4$ solution. The crystallized chlorophyll-protein was dissolved in 1 ml 0.1 M sodium phosphate/potassium phosphate buffer, pH 7.2 and recrystallized by means of dialysis against 90% satd. $(NH_4)_2SO_4$.

The chlorophyll-protein prepared from the leaves collected in the campus of Toho University formed two different shapes of crystal. The crystals shown in Fig. 1-1 are thin plates of parallelogram shape about 50 μm across and about 5 μm thick. The angles at the corners were 82 and 98°. The crystals in Fig. 1-2 were nearly cubic with sides of about 10 μm. Both of the crystalline shapes were, however, essentially hexahedrons with parallel faces. One of the two types of crystal was formed in respective preparations, even when the same procedure was employed. In twelve independent experiments, the shapes of Fig. 1-1 and 1-2 were formed ten times and twice, respectively. The origin of the difference in the crystalline shapes is not yet clarified.

The crystals of Fig. 1-3 were prepared from the plant cultivated in a green-house from seeds collected near Mono Lake. The crystals were wedge-shaped and different in shape from those in Fig. 1-1 and 1-2. The difference in crystalline shapes may be related to the difference in the chemical and physicochemical properties of chlorophyll-proteins, which will be published elsewhere.

French et al. [8] analyzed the absorption spectrum of chlorophyll in the chloroplast membrane, and presented a terminology 'chlorophyll forms'. We presume that distinctly different interactions between chlorophyll and

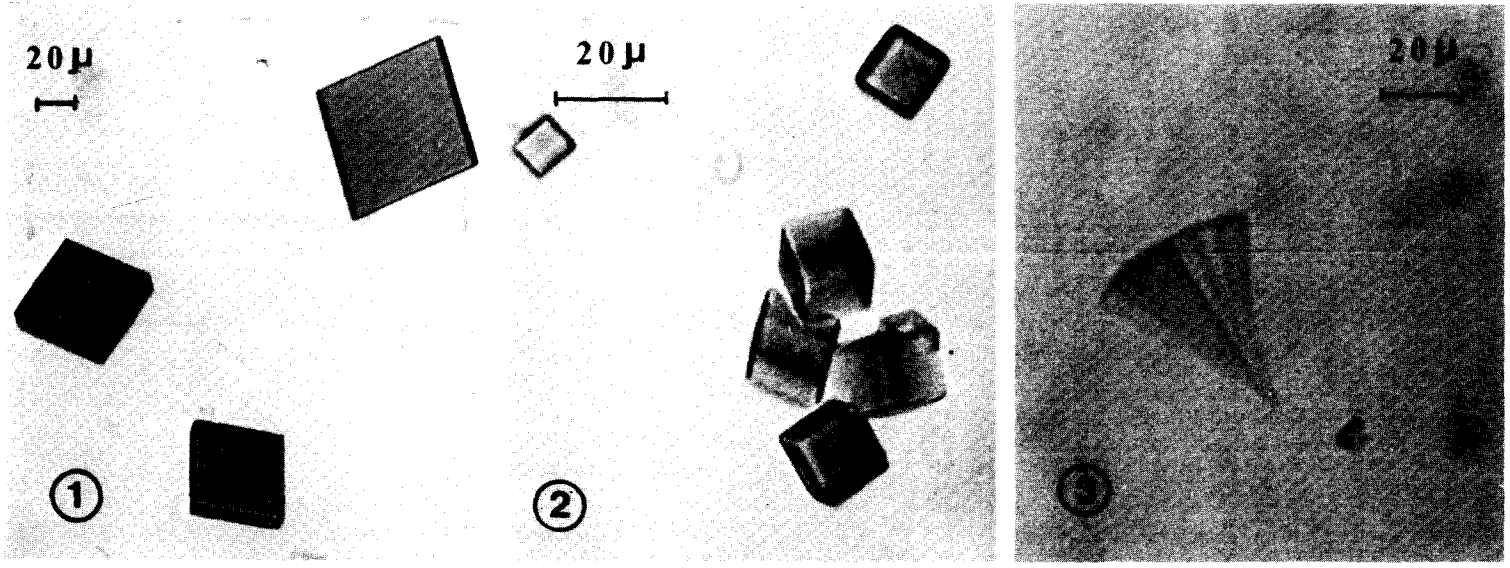

Fig. 1. Photomicrographs of crystals of chlorophyll-protein prepared from leaves of *L. virginicum*. Photographs 1 and 2 were from the plant collected in the campus of Toho University. Photograph 3 was from the plant cultivated in a green-house from seeds collected near Mono Lake.

protein molecules produce the different absorption spectra of chlorophyll forms. However, the modes of interactions have not yet been clarified at all. The chlorophyll-protein from *L. virginicum* is unique, since the red absorption band is located at a wavelength much shorter than those of chloroplasts. The analysis of the absorption spectrum in the curve-fitting method has revealed the occurrence of one chlorophyll *b* form, Cb650, and two chlorophyll *a* forms, Ca662 and Ca670, in this protein [9]. Thus, X-ray crystallographic analysis of the protein can be an appropriate model system to examine the modes of interactions which produce the different chlorophyll forms.

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