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Crystallization and preliminary X-ray analysis of a peridinin-chlorophyll *a* protein from *Amphidinium carterae*

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Crystals of a water-soluble ($M_r \approx 39\,000$) peridinin-chlorophyll *a* protein from *Amphidinium carterae* are reported. The crystals diffract to 2.2 Å and belong to a monoclinic (B2) and a triclinic (P1) space group. Spectra of the protein in the crystal and in solution are almost identical.

Antenna protein; Protein crystallization; X-ray diffraction

1. INTRODUCTION

Light-harvesting chlorophyll-protein complexes are typically membrane-bound in the eucaryotic algae and higher plants [1]. Dinoflagellates possess additionally a unique water soluble chlorophyll-protein which is readily liberated when the cells are disrupted [2]. This water-soluble pigment-protein contains chlorophyll *a* and peridinin only and has been designated peridinin-chlorophyll-protein (PCP). PCP has been purified from a number of dinoflagellates including *Amphidinium carterae* [3]. The molecular mass of the *Amphidinium* apoprotein is ≈32 000 Da and that of the holoprotein ≈ 39 000 Da and it contains 8–9 peridinin and two chlorophyll *a* molecules which are not covalently linked to the apoprotein [4]. The peridinin molecules occur as pairs of dimers in close proximity to a chlorophyll *a* molecule but each chlorophyll *a* molecule is sufficiently separated to preclude exciton coupling [5]. Radiation absorbed by the peridinin dimers is transferred with ≈100% efficiency to chlorophyll *a* [5]. The mechanism by which energy is transferred amongst PCP molecules or from these to the reaction centre complexes is not known. An insight into these problems should be obtained from the structural relationship of the chromophores with each other and the apoprotein. In this paper we report the crystallization of PCP from *Amphidinium carterae* together with some preliminary crystallographic data.

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Abbreviations: PCP, peridinin-chlorophyll-protein: PEG, poly(ethyleneglycol); SDS, sodium dodecyl sulfate

2. MATERIALS AND METHODS

Amphidinium carterae was grown in Provasoli's enriched sea water [6] at a light intensity of 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Cells were harvested by floccullation with 0.5 mM Al $K(SO_4)_2 . 12H_2O$ and washed with 50 mM tricine, 20 mM KCl, pH 7.5, and broken by a single passage through a French pressure cell at 67 MPa. Cell debris and membranes were removed by centrifugation at 25 000 × *g* and the supernatant subjected to ammonium sulphate fractionation. The fraction from 70–95% saturation contained the PCP. The PCP precipitate was equilibrated with 10 mM Na-acetate, pH 5.0, by passing it twice over a PD10 Sephadex column. PCP was further purified by cation exchange chromatography on CM Tris-acryl using a gradient of 0.0 to 0.5 M NaCl. The major part of the PCP eluted at ≈0.075 M NaCl. Fractions having an absorbance ratio A_{478nm}/A_{280nm} greater than 4.5 were pooled. PCP prepared as above was >95% homogenous as judged by SDS polyacrylamide gel-electrophoresis and HPLC size exclusion chromatography.

Electrophoretically pure PCP protein solutions were gel-filtrated using Sephacryl S-200 (Pharmacia, Fine Chem.) to analyse the aggregation behaviour. The column was equilibrated with 50 mM Tris-Cl, pH 7.5, 5 mM NaN_3 (indicated as standard buffer in the following) and calibrated with proteins of known molecular mass. PCP concentrations were estimated from $E^{0\,1\%}_{1cm} = 21.7$ at 476 nm [3].

Crystals were obtained by vapor diffusion technique, using PEG 10000. For crystallization, the PCP pigment-protein complex was concentrated to 24 mg/ml with a Amicon ultrafiltration cell equipped with a YM-10 membrane and dialysed against standard buffer. A presaturated protein solution was prepared by addition of a stock solution consisting of PEG 10000 and KCl in concentrations of 15% w/v and 2.4 M. The presaturated solution contained protein, PEG 10000, KCl, $MgCl_2$ and triethylammoniumphosphate in concentrations of 12 mg/ml, 7.5% w/v, 1.2 M, 10 mM and 2% w/v, respectively. Three reservoirs were made, containing PEG 10000 and KCl in concentrations of 15% w/v, 2.4 M; 12.5% w/v, 2.0 M; 10% w/v, 1.6 M, respectively. The presaturated solution was equilibrated against the reservoirs via the vapor phase.

Crystals were mounted in 1 mm glass capillaries (Müller, FRG) which were sealed. Photographs were taken on a precession camera (reciprocal lattice explorer: Stoe, Darmstadt, FRG), set at 125 mm crystal-to-film distance. The source of CuK_α X-rays was a rotating anode generator (Siemans, AG, FRG). The crystals were maintained at 11°C using a flow of cold air.

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The density of the crystals was determined with a linear gradient with density range from 0.9 to 1.5 g/cm^3 made from bromobenzene and xylene. The gradient was calibrated by measuring the refractive index vs the density of different bromobenzene/xylene mixtures. Using the refractive index of the thin layer within the gradient where the crystals were floating, the crystal density was determined.

For SDS polyacrylamide gel-electrophoresis the crystals were collected and washed free of dissolved protein with the above-mentioned stock solution by 4 centrifugation steps (Biofuge A, Heraeus). The pelleted crystals were dissolved in water and dialysed against a standard buffer for 20 h at 4°C. The protein and the dissolved crystals were solubilized with SDS containing buffer, analysed and stained with Coomassie R350 with a Phast-gel device (Pharmacia), following the instruction manual.

Spectra of thin rectangular crystals using polarized light were recorded on a home-built microspectrophotometer [7]. Thin crystals were obtained from a presaturated protein solution containing protein, PEG 1000 and $MgCl_2$ in concentrations of 5 mg/ml, 13% w/v and 15 mM, respectively. The presaturated solution was spread on thin cover slides and equilibrated with 20% w/v PEG 1000.

3. RESULTS AND DISCUSSION

In gel filtration experiments, the purified PCP protein eluted as a single band. Its molecular mass can be estimated by comparison with calibrating proteins as 40 000 Da indicating monomeric solutions. Crystalline plates for X-ray analysis grow within 5–10 days and have typical edge lengths of 0.7 × 0.7 × 0.15 mm (Fig. 1).

Precession photographs revealed two different space groups, a monoclinic space group B2 and a triclinic space group P1.

The lattice constants of the monoclinic form are a = 183 Å, b = 83 Å, c = 115 Å and γ = 111.3°. A precession photograph of the $h,0,l$ plane is shown in Fig. 2. A two-fold symmetry and systematic absences $h+l = 2n$ are seen.

The lattice constants of the triclinic space group are a = 117 Å, b = 108 Å, c = 78 Å, α = 72.9°, β = 69.8° and γ = 60.9°. The volume of the triclinic unit cell is calculated to be 794 000 Å^3 and is about half the volume of the monoclinic unit cell (1 627 420 Å^3).

In still photographs (Fig. 3) reflections were observed from both crystals form to 2.2 Å. The crystals were stable in the X-ray beam up to 50 h.

The density of the triclinic crystals was measured to be 1.1775 ± 0.0014 g/cm^3. The errors of measurement can be neglected. If the density of the solvent within the crystal is known, the mass of the protein in the unit cell can be calculated, using the known density of proteins (ϱ = 1.34 g/cm^3) [8] and the solvent density. The latter will be between the two extreme values for the density of water and of 1.2 M KCl (ϱ = 1.088 g/cm^3). The protein mass per unit cell therefore is between 227 500 Da and 334 000 Da corresponding to 6–9 PCP molecules. Assuming a similar density for both crystal forms, each of the 4 asymmetric units of the monoclinic form contains 3–5 PCP molecules.

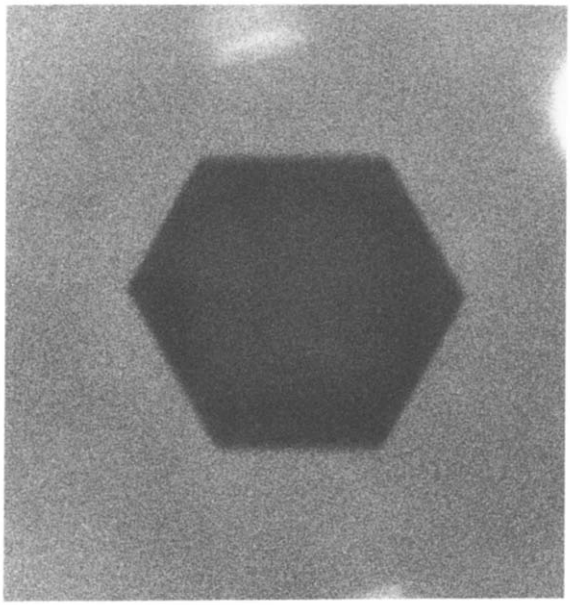

Fig. 1. Crystal of peridinin-chlorophyll-protein with edge lengths of 0.6 × 0.6 mm^2. The hexagonal habit is characteristic for the triclinic crystal form.

Gel-electrophoretic analysis of the washed crystals showed a single polypeptide band at an apparent molecular mass of 32 000 Da.

In Fig. 4 the vertical and horizontal polarized absorbance spectra of the crystallized PCP complex are shown. The horizontal absorbance is recorded along the short crystal edge and the vertical absorbance along the long crystal edge. The crystals show the strongest

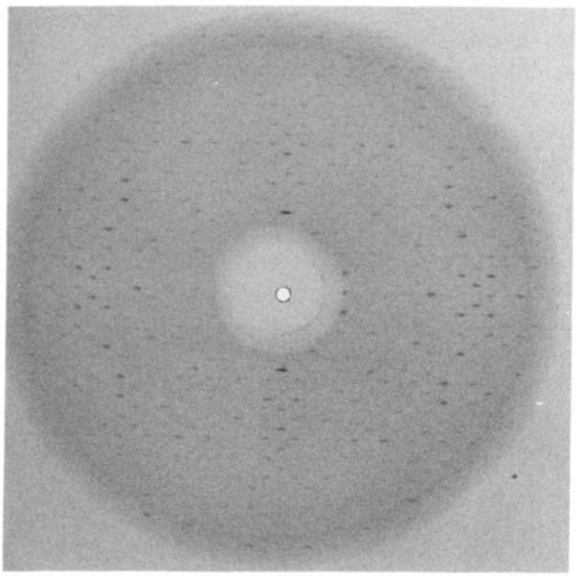

Fig. 2. Precession photograph of the monoclinic crystal form. The $h,0,l$ plane is seen with the two-fold symmetry axis along c (oriented horizontally in the photograph).

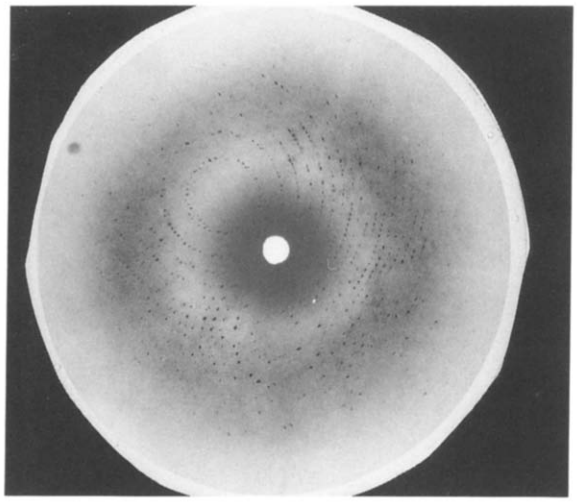

Fig. 3. Still photograph of triclinic crystal form. Diffraction spots extent to 2.2 Å.

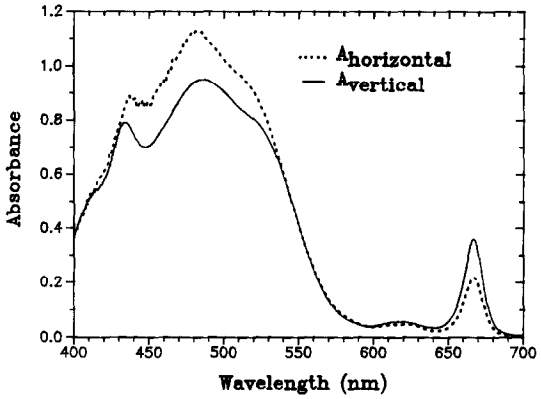

Fig. 4. Polarized absorbance spectra of thin, rectangular PCP crystals. The chlorophyll Q_y absorption band at 670 nm possesses the maximal dichroism (40%) in the crystal plane.

dichroism of 40% in the absorbance range of the Q_y transition of chlorophyll *a* at 670 nm.

The absorbance maximum at 485 nm is shifted by 9 nm to longer wavelengths, compared to the protein in solution [3]. By application of the linear correlation analysis [9] the dichroism of the two spectra between 400 and 550 nm was calculated (data not shown). The maximum of the dichroism (12%) was found at 510 nm. The absorbance spectra of the crystals indicate an almost native structure of the crystallized protein. The crystal data show, that PCP crystals were obtained which diffract to high resolution so that X-ray structure analysis seems to be possible. We intend to collect X-ray data from the present crystals as well as to look for other crystal forms.

So far only 3 structures of photosynthetic pigment-proteins are known. These are the bacteriochlorophyll *a* protein from *Prosthecochloris aestuarii* [10], the bacteriochlorophyll containing reaction center from purple bacteria [11,12] and the bilin containing cyanobacterial c-phycocyanin and phycoerythrocyanin [13,14]. Neither of the first two protein structures shows the arrangement of carotenoids together with chlorophylls at high resolution. The PCP protein crystals could help to elucidate the transfer of excitation energy from carotenoids to chlorophylls. In addition, the structure of the PCP protein is of interest as the protein functions as a special antenna protein, which transfers excitation energy to chlorophyll *a* molecules of the inner antennae surrounding the photosynthetic reaction centres.

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