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Crystallization of phycoerythrin 545 of *Rhodomonas lens* using detergents and unusual additives

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Abstract

Phycoerythrin 545 from the cryptomonad alga, *Rhodomonas lens*, has been crystallized under a wide variety of conditions. Although this type of photosynthetic light-harvesting protein is water soluble, detergents were always required for crystallization. The crystals were typically poorly ordered, or ordered in only two dimensions. However, crystals that were well-ordered in three dimensions could be obtained under two different conditions. Both used polyethylene glycol as precipitant and the detergent lauryldimethylaminoxide, but the additives that were critical for obtaining well-ordered crystals were propionamide in one case and Cs^+/Br^- in the other. Crystals obtained in the presence of propionamide have the space group $P2_12_12_1$, with cell constants of a = 85.6 Å, b = 108.2 Å, and c = 131.0 Å, and contain two dimers [i.e., $2 \times (\alpha_2\beta_2)$] in the asymmetric unit. They show diffraction to at least 3.0 Å resolution. The crystals grown with Cs^+/Br^- are nearly isomorphous. Both types of crystals show intense, strongly polarized fluorescence, suggesting that energy transfer in the crystals is highly efficient. This should provide a basis for quantitative investigation of the role of exciton interactions in energy transfer in cryptomonad phycobiliproteins.

Keywords: Cryptophyceae; detergent; light-harvesting protein; photosynthesis; phycobiliprotein; propionamide; protein crystal; X-ray diffraction

Photosynthetic light-harvesting proteins contain pigments that absorb photons and transfer excited-state energy to photosynthetic reaction centers, where the captured energy initiates an excited-state oxidation reaction of a specialized set of pigments that leads to charge separation across the photosynthetic membrane. In most organisms, light harvesting is performed mainly by chlorophyll-containing membrane proteins. However, cyanobacteria, red algae, and cryptomonad algae possess additional light-harvesting proteins, called phycobiliproteins, which are water-soluble and contain covalently bound open-chain tetrapyroles as pigments (MacColl & Guard-Friar, 1987; Glazer, 1989). These proteins absorb strongly between about 470 and 650 nm, where chlorophyll absoprtion is weak.

The phycobiliproteins of the cyanobacteria and the red algae have been well characterized. There are four main types: phycoerythrin ($A_{max} \approx 550$ nm), phycoerythrocyanin ($A_{max} \approx 575$ nm), phycocyanin ($A_{max} \approx 620$ nm), and allophycocyanin ($A_{max} \approx 650$ nm). They are all similar in that their monomeric units consist of two subunits, called α and β, which are highly homologous to each other, and which are highly homologous between different types of proteins. Each subunit has a molecular weight of about 16–20 kDa and contains 1–3 covalently bound pigments. X-ray crystal structures (Schirmer et al., 1985; Deurring et al., 1990; Ficner et al., 1992; Brejc et al., 1995) show that each subunit consists of eight α-helices folded in a manner similar to the globin fold. The monomers assemble to yield ring-like trimeric disks of about 110 Å × 30 Å that contain a central hole of about 35-Å diameter. The pigments are bound to the protein in extended conformations, which is important for tuning their spectral and excited-state properties. In vivo, phycobiliproteins aggregate along with linker proteins to form complicated, highly ordered, supramolecular structures, known as phycobilisomes, which extend from the stromal surface of the thylakoid membrane.

In contrast to the proteins of the cyanobacteria and of the red algae, the cryptomonad phycobiliproteins are unusual in several ways (MacColl & Guard-Friar, 1987; Glazer & Wedemayer, 1995). First, each species of cryptomonad contains only one type of phycobiliprotein—either phycoerythrin or phycocyanin. Second,

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Abbreviations: A_{max}, absorption maximum; β-ME, β-mercaptoethanol; bis-Tris, bis-(2-hydroxyethyl)imino-tris-(hydroxymethyl)methane; DEAE-cellulose, diethylaminoethyl-cellulose; EDTA, ethylenediaminitetraacetic acid; HEPES, N-(2-hydroxyethyl)-piperazine-N′-(2-ethane-sulfonic acid); IEF, isoelectric focusing; LDAO, lauryldimethylaminoxide; PC645, phycocyanin 645; PE545, phycoerythrin 545; PEG, polyethylene glycol; UDAO, undecyldimethylaminoxide; V_M, Matthews parameter.

the proteins are found on the lumenal rather than the stromal side of the thylakoid membrane. Third, the proteins form dimers, rather than trimers or hexamers, where the β-subunits are similar in size to β-subunits of the more well-known phycobiliproteins, but the α-subunits are only about half the size. Although cryptomonad β-subunits shows approximately 70% sequence identity with β-subunits of the red algae, cryptomonad α-subunit sequences show only about 20% sequence similarity to α-subunits of non-cryptomonads (Sidler et al., 1990). Finally, cryptomonad phycobiliproteins contain several unusual bilins not found in non-cryptomonad proteins (Wedemayer et al., 1992), and their CD spectra show unusual features that might indicate strong excitonic coupling between pigments (Jung et al., 1980; MacColl et al., 1994).

Crystals of a cryptomonad phycobiliprotein were first reported in 1984 by Morriset et al., who crystallized phycocyanin 645 (PC645) from *Chroomonas* species. Although a structure solution was not possible at the time, two aspects of the work were particularly noteworthy. First, although PC645 is water soluble and does not require detergent for isolation, the detergent benzalkonium chloride was strictly required for obtaining crystals; Bode and Schirmer (1985) showed that detergent was tightly bound in the crystals. Second, the crystals did not show crystallographic threefold rotational symmetry, in stark contrast to crystals of other types of phycobiliproteins from cyanobacteria and red algae. To extend these studies, efforts were continued with several different cryptomonads; the most promising results, which were obtained for PE545 of *Rhodomonas lens*, are reported here.

Results

During early stages of screening, several factors favored the growth of crystals. These were: PEG 4000 or 6000, Tris, imidazole, or HEPES buffers, pH range about 8–6, $CaCl_2$ or Li_2SO_4, LDAO, and isopropanol. Even though large crystals could be grown from these conditions (up to about 1.2 × 0.3 × 0.2 mm), the diffraction was highly anisotropic, with diffraction to roughly 4 Å normal to the broadest face of the crystals, but to only about 8 Å from crystals oriented perpendicular to this direction. Under inspection with a microscope, the crystals appeared to be poorly ordered stacks of thin plates. During later stages in screening, two paths led to crystals with improved three-dimensional diffraction properties.

One successful strategy was to continue using essentially the same precipitants, buffers, and salts, but to substitute for isopropanol with a variety of small polar organic molecules roughly resembling isopropanol in size (at 1–5%, v/v), including other alcohols, amines, mixed amino-alcohols, amides, ketones, and sulfoxide. Almost all of the additives yielded crystals, and many had dramatic effects on the crystal habits. Photos of some examples of crystals from later stages of screening (which included a variety of ions as well—see below) are shown in Figure 1A–F. In general, a trend in the relative improvement of diffraction in three dimensions can be summarized as follows:

propionamide

> (butyramide; DMSO) > glycerol

> (acetamide; ethylene glycol; propandiols; isopropanol)

≫ other mono-alcohols.

Although the better of the above additives yielded crystals that were usually at least moderately ordered, various amines and hydroxyacetone had the tendency to yield crystals that were badly twinned or disordered.

The best results were achieved with propionamide (Table 1). Crystals suitable for structural studies were generated as follows: using 24-well sitting drop plates, a 400-μL reservoir solution containing 0.1 M imidazole, pH 6.8, 0.2 M Li_2SO_4, 3% propionamide, 10% PEG 4000, and 0.02% NaN_3 was equilibrated with a sitting drop made by mixing 4 μL of reservoir solution with 4 μL of PE545 at 15 mg/mL in 10 mM bis-Tris, pH 6.0, 2 mM β-ME, 0.02% NaN_3, and then adding 1 μL of an LDAO stock solution at 0.8 mg/mL. This yields an LDAO concentration of about 0.01% (0.4 mM) in the initial drop volume, and presumably about 0.02% in the equilibrated drop volume. Under equilibration at 5 °C, crystals appeared after about 1 week and grew to a size of about 0.8 mm × 0.3 mm × 0.2 mm in about 2 weeks to 1 month (Fig. 2A,B). The diffraction from these crystals was observed to at least 3.0-Å resolution along all three major axis. The crystals were fairly stable to X-ray irradiation, and an initial data set was collected from a single crystal at 5 °C. The reflections were indexed in an orthorhombic crystal system having cell constants of a = 85.6 Å, b = 108.2 Å, and c = 131.0 Å, and the data were 87% complete to 3.4-Å resolution (Table 2). Systematic absences for odd reflections along all three major axes were observed in the oscillation data and in screenless precession photos, indicating that all three axes are screw axes, at least as determined at low resolution, i.e., the space group is apparently $P2_12_12_1$.

V_M calculations (Matthews, 1968) suggest that there are most likely two dimers in the asymmetric unit (V_M = 2.78 Å^3/Da; volume fraction of solvent = 56%). The Patterson self-rotation function (κ = 180°) did not show any obvious peaks resulting from a noncrystallographic symmetry axis. However, inspection of the Harker sections revealed a strong peak at position (0.50, 0.31, 0.00) on Harker plane u = 1/2 with a height of 38% of the origin peak (Fig. 3) resulting from a local symmetry axis parallel to the crystallographic a-axis (Eagles et al., 1969; Epp et al., 1971). The presence of this peak and its crystallographically related mate at (0.50, 0.69, 0.00) indicates that the local symmetry axis results from a local twofold rotation, which is positioned at (x, 0.31/2, 0) relative to the crystallographic screw axis along a.

Besides the extinctions resulting from the crystallographic screws along the major axis, additional systematic extinctions or near extinctions were observed in the $h0l$ zone, where odd layers of h are absent or extremely weak, i.e., h = 2n (Fig. 4). This extinction pattern was seen both in oscillation images and in screenless precession photographs. It indicates a molecular packing in layers perpendicular to a and with a spatial separation of $a/2$. This extinction pattern, coupled with the local symmetry peaks on the Harker line at (1/2, v, 0), might indicate that the crystal packing posseses $a/2$ pseudo-glide plane symmetry normal to the b-axis, although this must be confirmed by model building.

The second strategy that yielded well-diffracting crystals was to screen similar to above, but to also radically vary the compliment of ions. Again, crystals were produced under most conditions, with or without organic additives (see Fig. 1A–F for some examples). A major improvement in diffraction was seen in crystals obtained by using a combination of Cs^+ and Br^- in the absence of an organic additive (Table 1). These crystals have the appearance of long flat orthorhombs, they are nearly isomorphous with and show the same

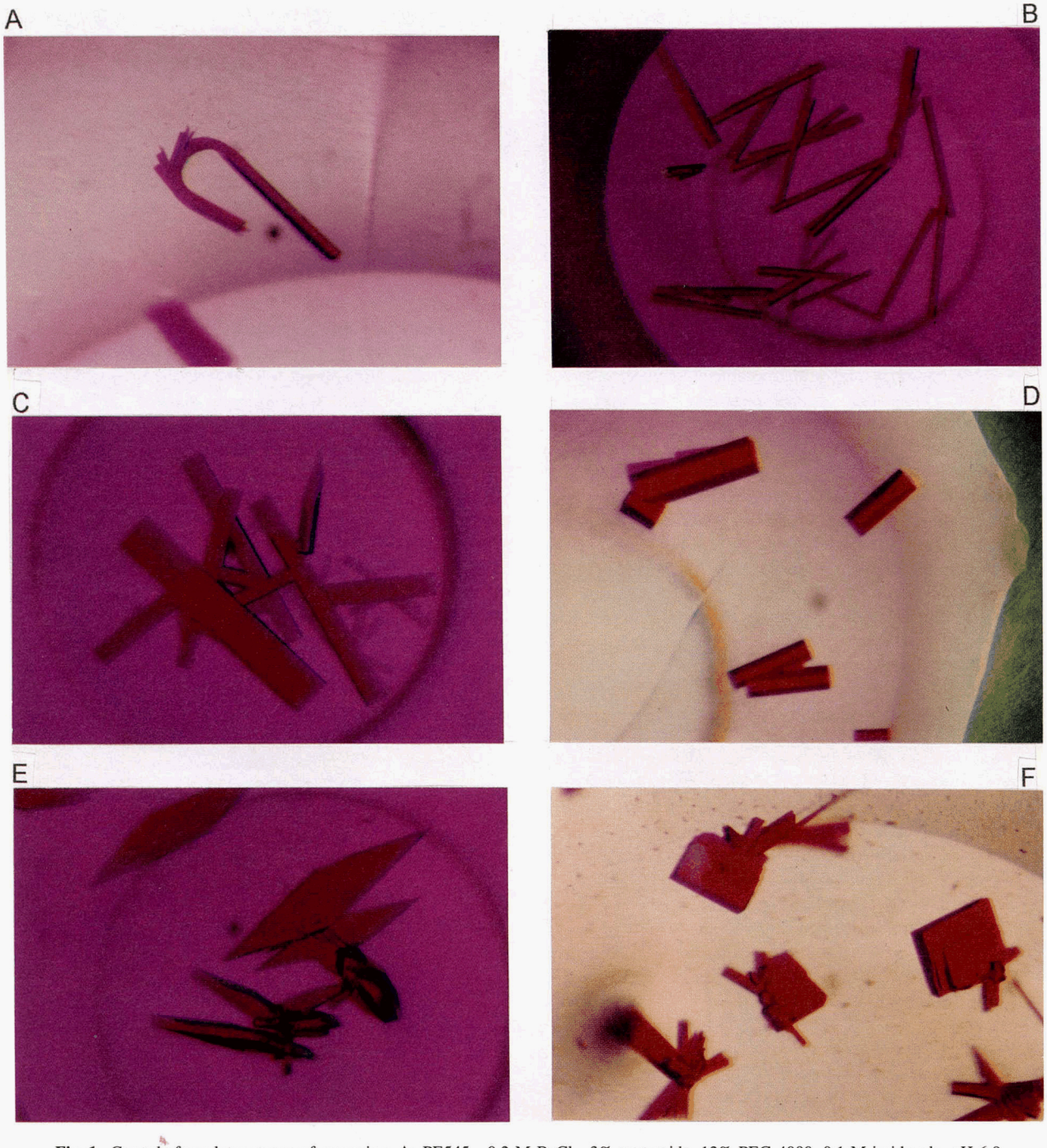

Fig. 1. Crystals from later stages of screening. **A:** PE545—0.2 M $BaCl_2$, 3% acetamide, 12% PEG 4000, 0.1 M imidazole, pH 6.9, 0.02% LDAO, 16 °C, 50×. **B:** PE545—0.1 M CsCl, 0.1 M KBr, 3% propionamide, 12% PEG 4000, 0.1 M imidazole, pH 6.9, 0.02% LDAO, 5 °C, 20×. **C:** PE545—0.2 M Li_2SO_4, 3% butyramide, 1% L-lysine, 12% PEG 4000, 0.1 M HEPES, pH 6.9, 0.02% LDAO, 5 °C, 30×. **D:** PE545—0.2 M CsCl, 2% ethylendiamine, 2% gylcerol, 10% PEG 6000, 0.1 M imidazole, pH 7.3, 0.02% UDAO, 16 °C, 25×. **E:** PE545—0.2 M Li_2SO_4, 3% diaminopropane, 1% ethylenglycol, 12% PEG 6000, 0.1 M imidazole, pH 6.8, 0.02% LDAO, 5 °C, 30×. **F:** PE545—0.02 M $LaCl_3$, 0.2 M NaCl, 3% DMSO, 1% glycerol, 10% PEG 4000, 0.1 M HEPES, pH 6.7, 0.02% UDAO, 16 °C, 40×.

extinction pattern as the crystals obtained with propionamide, and they are only slightly less stable in the X-ray beam.

With most conditions, LDAO was the detergent that gave the best results. However, the 11-mer, undecyldimethylaminoxide, and the 13-mer, tridecyldimethylaminoxide, were also capable of yielding crystals with moderate diffraction properties. In all cases, it was essential to provide detergent in the mounting buffers because cracks appeared otherwise.

Even though the samples used in crystallization were partially enriched in certain isoforms, IEF gels run on dissolved crystals showed

Table 1. *PE545 crystal properties*

Crystallization conditions:	0.1 M imidazole, pH 6.8, 0.2 M Li_2SO_4, 3% propionamide, 10% PEG 4000, 0.02% NaN_3, 0.02% LDAO, 15 mg/mL PE545, sitting drop at 5 °C	0.1 M HEPES, pH 6.8, 0.1 M CsCl, 0.1 M KBr, 10% PEG 4000, 0.02% NaN_3, 0.02% LDAO, 15 mg/mL PE545, sitting drop at 21 °C
Space group:	$P2_12_12_1$	$P2_12_12_1$
Cell constants:	a = 85.6 Å, b = 108.2 Å, c = 131.0 Å	a = 83.3 Å, b = 105.7 Å, c = 127.4 Å
V_M:	2.8 Å^3/Da for 2 × $(\alpha_2\beta_2)$/a.u.	2.6 Å^3/Da for 2 × $(\alpha_2\beta_2)$/a.u.
Diffraction limit:	At least 3.0 Å	At least 3.0 Å

several bands. Comparison to IEF gels run on different fractions from a DEAE-cellulose column suggested that some of the IEF bands were indicative of dissociated subunits and probably result from crystal decay or denaturation of the complex in the gel. SDS gels showed the normal two α and one β bands. There were sometimes very weak contaminant bands of higher molecular weight, but these did not appear to be found consistently from crystal to crystal.

All of the well-ordered crystals as well as the moderately ordered crystals showed strongly polarized fluorescence (Fig. 5). It was confirmed that the polarization of the detected light was indeed mainly a result of polarized fluorescence and not simply an artifact of the dichroic and birefringent properties of the crystals by placing crystals between crossed polarizers in a fluorescence microscope equipped with time-resolved detection (fluorescence microscope courtesy of Dr. Gerard Marriott, Abt. für Zellbiologie, Max-Planck-Institut für Biochemie).

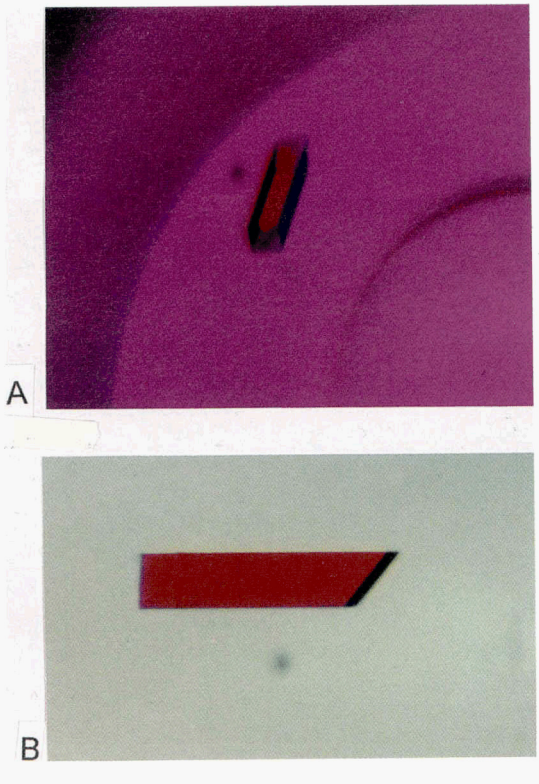

Fig. 2. A: PE545 crystal grown with 0.1 M imidazole, pH 6.8, 0.2 M Li_2SO_4, 3% propionamide, 10% PEG 4000, 0.02% NaN_3, 0.02% LDAO at 5 °C (as in Table 1). **B:** Same crystal viewed in a drop of mother liquor on a glass microscope slide.

Discussion

The arrangement of cryptomonad phycobiliproteins in the thylakoid lumen and the detailed nature of their connection to the membrane are not well understood. Mörschel and Wehrmeyer (1979)

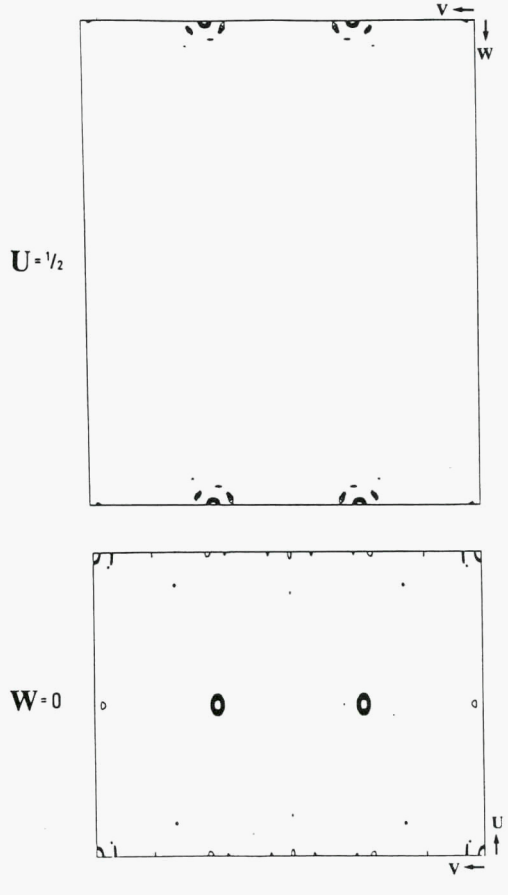

Fig. 3. Harker section at u = 1/2 and intersecting Patterson layer at w = 0. Calculated with data ranging from 8.0 to 3.5 Å resolution. Contours are plotted in steps of 1σ, and only those that are greater than 3σ above mean are shown. Height of the peak at (0.50, 0.31, 0.00) is 38% of that of the origin peak. The next highest peak in the map has a height that is 9% of the origin peak.

Table 2. *Data collection statistics*[a]

Possible # unique reflections	Measured # unique reflections	Resolution per shell (Å)	Completeness per shell (%)	Total resolution (Å)	Total completeness (%)
4,454	4,156	∞–5.43	93.3	∞–5.43	93.3
4,225	4,005	5.43–4.31	94.8	∞–4.31	94.0
4,221	3,945	4.31–3.76	93.5	∞–3.76	93.8
4,178	2,839	3.76–3.42	68.0	∞–3.42	87.5

[a]A total of 29,642 reflections was measured, which reduced to 14,945 unique reflections.

$$R_{merge} = 9.92\%, \text{ where } R_{merge} = \frac{\sum|I(h) - \langle I(h)\rangle|}{\sum I(h)}$$

and $I(h)$ is the observed intensity of reflection h, $\langle I(h)\rangle$ is the mean intensity for all reflections h.

have interpreted electron microscope images as providing evidence for small columns of biliproteins extending out from the membrane, and Lichtlé et al. (1987) have isolated particles that apparently show columns of phycoerythrin extending from thylakoid vesicles. Electron microscopy using gold-labeled antibodies indicates that PE545 in *R. lens* appears to be closely associated with the membrane (Ludwig & Gibbs, 1989). The properties of the PE545 crystals obtained here and in the study of Morriset et al. (1984) suggest that cryptomonad phycobiliproteins contain a significantly large hydrophobic surface, and that they may possibly bind directly to the thylakoid membrane. However, the presence of dramatically different crystal habits depending on which organic or ionic additives were used suggests that both organic and ionic additives might specifically bind in the crystals; if so, specific binding could mimic the nature of possible *in vivo* interactions, such as the binding to putative linker proteins or ion-mediated binding to components of the thylakoid membrane.

Addressing the question of how propionamide and Cs^+/Br^- improve the diffraction quality of the crystals, additional possibilities besides specific binding can also be considered, including potential small amphiphile effects for propionamide (Michel, 1983). However, the similarity of the crystal forms obtained in this study using either propionamide or Cs^+/Br^- might suggest a more general effect of these components, possibly related to stability and/or chaotropic effects. Besides physico-chemical obstacles to obtaining well-ordered crystals, a major difficulty in crystallizing cryptomonad phycobiliproteins is that native samples are usually inhomogeneous. At least some of the heterogeneity apparently results from having different combinations of similar but genetically distinct subunits, which yields a mixture of homodimers and heterodimers with isoelectric points that are close together (Hiller & Martin, 1987; Jenkins et al., 1990). Clean separation of isoforms suitable for crystallization is rendered difficult by instability of the proteins at pH values differing much from neutrality.

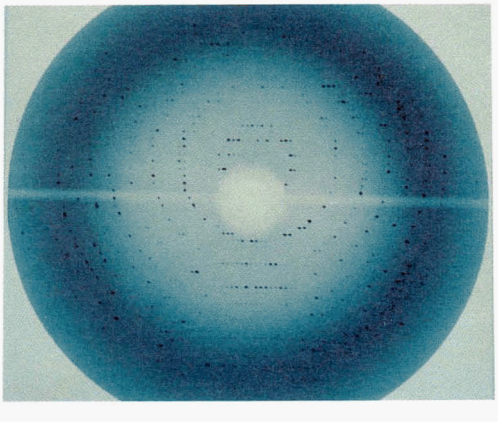

Fig. 4. Diffraction from a crystal grown with propionamide as in Figure 2A, as viewed nearly along b^* with a MAR image plate detector, showing the $h01$ zone. Notice that the $h = 2n + 1$ layers in this zone are very weak.

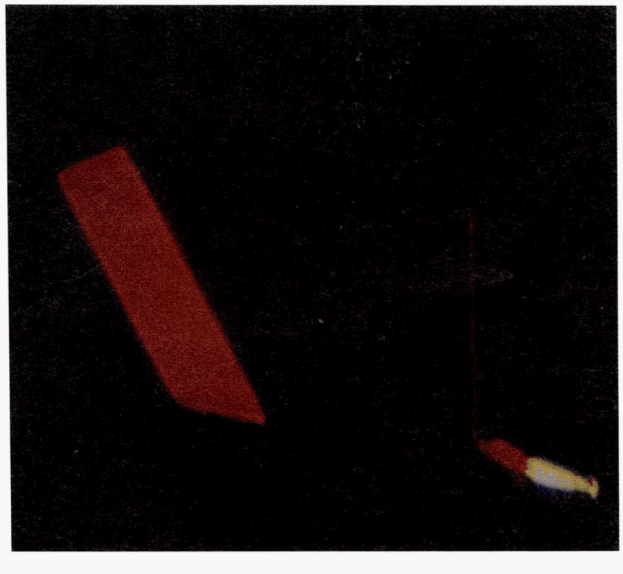

Fig. 5. Two crystals grown as in Figure 2A, as seen between crossed polarizers. The crystal on the left shows bright orange fluorescence; the crystal on the right is essentially devoid of emission (except as a result of a piece of scattering material that helps the viewer to visualize a part of the crystal).

PE545 is particularly interesting from the functional point of view, because its Förster overlap integral with chlorophyll is about a factor of 5 smaller than is typical for other donor–acceptor pairs (MacColl & Berns, 1978), yet it is highly efficient in energy transfer to the membrane. From the apparent polarized fluorescence of the crystals, vectorial energy transfer within the crystals seems to be highly efficient. Comparison of the fluorescence polarization of the crystals to the final structure may indicate whether exciton interactions contribute appreciably to the unusual CD spectra of the cryptomonad proteins, and to the mechanism(s) by which energy is transferred rapidly and efficiently (Jung et al., 1980; Holzwarth, 1991; MacColl et al., 1994).

Materials and methods

Cell culture

Generous instruction in the growth of cryptomonads was kindly provided by C. Schimeck and Prof. W. Wehrmeyer; starting cultures of *R. lens* were generously provided by Dr. R. MacColl. *R. lens* was grown at 15 °C with 600 lux of continuous-illumination white light on modified DV media (MacColl & Guard-Friar, 1983), bubbled with approximately 5% CO_2.

Protein preparation

Cells (12–15 L) were harvested by centrifugation at 1,000 × *g* at 4 °C. Pellets were resuspended to a volume of about 200 mL with a buffer solution containing 20 mM bis-Tris, pH 6–7, 2 mM EDTA, 2 mM β-ME, and 0.02% NaN_3, and then frozen at −80 °C. Later, the cells were thawed and centrifuged at 31,000 × *g* at 4 °C for 1 h to pellet the membranes. The supernatant containing the biliproteins was brought to 40% saturation with ammonium sulfate, stored overnight at 5 °C, and centrifuged at 31,000 × *g* to pellet residual particulate material. The supernatant solution was then brought to 80% saturation with ammonium sulfate, stored overnight at 5 °C, and centrifuged at 31,000 × *g* to pellet the phycobiliproteins. The pellets were resuspended to about 100–200 mL with 10 mM bis-Tris, pH 6.0, 2 mM EDTA, 2 mM β-ME, 0.02% NaN_3. After another precipitation cycle, protein was resuspended with the same solution, desalted with PD-10 columns (Pharmacia), and purified further. MacColl et al. (1992) observed six isoforms for PE545 with pI values ranging from 4.9 to 6.7, and the two major isoforms having pI values of about 6.5 and 5.2. In the preparations here, there were a similar number of isoforms, but with the two major isoforms having pIs of roughly 5.5 and 5.3. These differences may reflect differences in sample preparation, growth conditions, and/or methods of IEF. Fractions of PE545 enriched in these two isoforms were obtained by isocratic elution at 0.8 mL/min of a Whatman DE-52 column (20 cm × 1 cm) equilibrated with 10 mM bis-Tris buffer, pH 6.0, 2 mM EDTA, 2 mM β-ME, 0.02% NaN_3 at 5 °C. The first major fluorescent band off the column, which was enriched in the two isoforms, was precipitated with ammonium sulfate, desalted, and concentrated to about 15 mg/mL for crystallization. During isolation and handling, proteins were kept in darkness or dim light. For analysis of the proteins, IEF gels were SERVALYT Precotes 3–10 run on a Pharmacia PHAST gel system. SDS gels were 14% or 17% in polyacrylamide, run on a PHAST gel system.

Crystallization

Crystallization was originally screened by using vapor diffusion with conditions based on Morriset et al. (1984), as well as on those of Jancarik and Kim (1991), and by using a variety of detergents. LDAO, propionamide, and PEG were obtained from Fluka. Reported pH values were measured at 21 °C after mixing stock solutions containing buffer, precipitant, salt, and additive, prior to mixing with aliquots of protein and detergent stocks for making drops. Crystallization dishes were kept in the dark.

Data collection and analysis

Diffraction data were collected by using a MAR Research image plate mounted on a Rigaku RU200 rotating anode generator. The reflections were indexed and integrated using MOSFLM (Leslie, 1992), data were merged and scaled using ROTAVATA/AGROVATA (CCP4, 1994), and Patterson and self-rotation functions were calculated using PROTEIN (Steigemann, 1974).

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References

Bode W, Schirmer T. 1985. Determination of the protein content of crystals formed by *Mastigocladus laminosus* C-phycocyanin, *Chroomonas* spec. Phycocyanin-645 and modified human fibrinogen using an improved ficoll density gradient method. *Biol Chem Hoppe-Seyler 366*:287–295.

Brejc K, Ficner R, Huber R, Steinbacher S. 1995. Isolation, crystallization, crystal structure analysis and refinement of allophycocyanin from the cyanobacterium *Spirulina platensis* at 2.3 Å resolution. *J Mol Biol 249*:424–440.

CCP4. 1994. Collaborative Computational Project, Number 4. The CCP4 suite: Programs for protein crystallography. *Acta Crystallogr D 50*:760–763.

Deurring M, Huber R, Bode W, Ruembeli R, Zuber H. 1990. Refined three-dimensional structure of phycoerythrocyanin from the cyanobacterium *Mastigocladus laminosus* at 2.7 Å. *J Mol Biol 211*:633–644.

Eagles PAM, Johnson LN, Joynson MA, McMurray CH, Gutfreund H. 1969. Subunit structure of aldolase: Chemical and crystallographic evidence. *J Mol Biol 45*:533–544.

Epp O, Steigemann W, Formanek H, Huber R. 1971. Crystallographic evidence for the tetrameric subunit structure of L-aspariginase from *Escherichia coli*. *Eur J Biochem 20*:432–437.

Ficner R, Lobeck K, Schmidt G, Huber R. 1992. Isolation, crystallization, crystal structure analysis and refinement of B-phycoerythrin from the red alga *Porphyridium sordidum* at 2.2 Å resolution. *J Mol Biol 228*:935–950.

Glazer AN. 1989. Light guides. *J Biol Chem 264*:1–4.

Glazer AN, Wedemayer GJ. 1995. Cryptomonad biliproteins—An evolutionary perspective. *Photosynth Res 46*:93–105.

Hiller RG, Martin CD. 1987. Multiple forms of a type I phycoerythrin from a *Chroomonas* sp. (Cryptophyceae) varying in subunit composition. *Biochim Biophys Acta 923*:98–102.

Holzwarth AR. 1991. Structure–function relationships and energy transfer in phycobiliprotein antennae. *Physiol Plant 83*:518–528.

Jancarik J, Kim SH. 1991. Sparse matrix sampling: A screening method for crystallization of proteins. *J Appl Crystallogr 24*:409–411.

Jenkins J, Hiller RG, Speirs J, Godovac-Zimmermann J. 1990. A genomic clone encoding a cryptophyte phycoerythrin α-subunit. *FEBS Lett 273*:191–194.

Jung J, Song PS, Paxton RJ, Edelstein MS, Swanson R, Hazen EE Jr. 1980. Molecular topography of the phycocyanin photoreceptor from *Chroomonas* species. *Biochemistry 19*:24–32.

Leslie AGW. 1992. Recent changes to the MOSFLM package for processing film and image plate data. *Joint CCP4 and ESF-EACBM Newsletters on Protein Crystallography 26*.

Lichtlé C, Duval JC, Lemoine Y. 1987. Comparative biochemical, functional and ultrastructural studies of photosystem particles from a Cryptophycea: *Cryptomonas rufescens*; isolation of an active phycoerythrin particle. *Biochim Biophys Acta 894*:76–90.

Ludwig M, Gibbs SP. 1989. Localization of phycoerythrin at the lumenal surface of the thylakoid membrane in *Rhodomonas lens*. *J Cell Biol 108*:875–884.

MacColl R, Berns DS. 1978. Energy transfer studies on cryptomonad biliproteins. *Photochem Photobiol 27*:343–349.

MacColl R, Guard-Friar D. 1983. Phycocyanin 612: A biochemical and photophysical study. *Biochemistry 22*:5568–5572.

MacColl R, Guard-Friar D. 1987. *Phycobiliproteins*. Boca Raton, Florida: CRC Press.

MacColl R, Guard-Friar D, Williams EC. 1992. Spectroscopic studies on phycoerythrin 545, its bilins, and its isoproteins. *J Luminescence 51*:21–28.

MacColl R, Lam I, Choi CY, Kim J. 1994. Exciton splitting in phycoerythrin 545. *J Biol Chem 269*:25465–25469.

Matthews BW. 1968. Solvent content of protein crystals. *J Mol Biol 33*:491–497.

Michel H. 1983. Crystallization of membrane proteins. *Trends Biochem Sci 8*:56–59.

Morriset W, Wehrmeyer W, Schirmer T, Bode W. 1984. Crystallization and preliminary X-ray diffraction data of the cryptomonad biliprotein phycocyanin-645 from *Chroomonas* spec. *Arch Microbiol 140*:202–205.

Mörschel E, Wehrmeyer W. 1979. Elektronenmikroskopische Feinstrukturanalyse von nativen Biliproteidaggregaten und deren räumliche Ordnung. *Ber Deutsch Bot Ges 92*:393–402.

Schirmer T, Bode W, Huber R, Sidler W, Zuber H. 1985. X-ray crystallographic structure of the light-harvesting biliprotein C-phycocyanin from the thermophilic cyanobacterium *Mastigocladus laminosus* and its resemblance to globin structures. *J Mol Biol 184*:257–277.

Sidler W, Nutt H, Kumpf B, Frank G, Suter F, Brenzel A, Wehrmeyer W, Zuber H. 1990. The complete amino-acid sequence and the phylogenetic origin of phycocyanin-645 from the cryptophytan alga *Chroomonas* sp. *Biol Chem Hoppe-Seyler 371*:537–547.

Steigemann W. 1974. Die Entwicklung und Anwendung von Rechenverfahren und Rechenprogrammen zur Strukturanalyse von Proteinen am Beispiel des Trypsin-Trypsininhibitor Komplexes, des freien Inhibitors und der L-Asparaginase [thesis]. Technischen Universität München.

Wedemayer GJ, Kidd DG, Wemmer DE, Glazer AN. 1992. Phycobilins of cryptophycean algae. *J Biol Chem 267*:7315–7331.