THE QUATERNARY STRUCTURE OF A UNIQUE PHYCOBILIPROTEIN: B-PHYCOERYTHRIN FROM *PORPHYRIDIUM CRUENTUM*

C. Abad-Zapatero, J. L. Fox
Department of Zoology

and

M. L. Hackert
Clayton Foundation Biochemical Institute
Department of Chemistry
University of Texas
Austin, Texas 78712

Received July 19, 1977

Summary: B-Phycoerythrin, from the unicellular red alga *Porphyridium cruentum*, was crystallized in the rhombohedral space group R3 with a=111.0Å and α=116.8° or A=B=189.1Å and C=60.1Å and γ=120°. Density measurements on the crystals indicate that the hexagonal unit cell can accommodate three cylindrical molecules, 109Å in diameter and 60Å in height, each of approximately 275,000 daltons. The crystallographic symmetry of the unit cell requires at least 3-fold symmetry for the particle. However, the particle stoichiometry has been reported as $(\alpha\beta)_6\gamma$ and this composition is also supported by SDS gel electrophoresis on the crystalline protein. These results are discussed in light of preliminary model calculations on the quaternary structure of B-phycoerythrin.

INTRODUCTION

Phycobiliproteins are a group of macromolecules that act as accessory photosynthetic pigments in prokaryotic (blue-green) as well as eukaryotic (red) algae. (For recent reviews, see 1-3). Three major classes of accessory pigments, the red phycoerythrins and the blue phycocyanins and allophycocyanins, have been characterized according to their absorption and fluorescence spectra. The prosthetic groups are all open chain tetrapyrroles covalently bound to the protein moiety. All of them, regardless of their origin or type of chromophore, appear to be descendents from a common ancestral protein (4).

Three types of phycoerythrins have been recognized according to their absorption spectra (5): C-phycoerythrins exhibiting a long wavelength absorption peak at approximately 565 nm; B-phycoerythrins having an additional peak at 545 nm and a shoulder at 498 nm; and R-phycoerythrins with maxima at 567, 538 and 498 nm.

All four major phycobiliproteins in the unicellular red alga *P. cruentum* are associated with organized macromolecular aggregates called phycobilisomes attached to the outer surface of the photosynthetic lamellae (6-10). Native

ISSN 0006-291X

B-phycoerythrin from *P. cruentum* was initially reported to have a molecular weight of 265,000 daltons by Sephadex G-200 gel filtration or 280,000 daltons by polyacrylamide gel electrophoresis (7). These data are consistent with the disc-like aggregate characterized by electron microscopy as a particle 115Å in diameter and 64Å in height (6). More recent experiments with the B-phycoerythrin from the same organism yield chemical and spectroscopic data consistent with a subunit structure composed of three dissimilar subunits, α and β of 17,500 daltons each, and γ of 30,200 daltons (11). Molecular weight and amino acid composition arguments led Glazer et al. to propose an $(\alpha\beta)_6\gamma$ subunit structure for the native B-phycoerythrin of molecular weight 240,000.

We present here preliminary crystallographic data on B-phycoerythrin from the red alga *P. cruentum* and also report the results of model calculations on the quaternary structure of the B-phycoerythrin oligomer. A tentative model for the electron density distribution in the oligomer is proposed which is consistent with the hk0 diffraction pattern to 20Å resolution. The possible arrangements of subunits corresponding to this electron density distribution are discussed on the basis of current biochemical data.

METHODS

Crystals of B-phycoerythrin were grown in microdiffusion cells (12) at a protein concentration of 4 mg/ml. Crystals of prismatic habit and hexagonal cross-section are obtained by dialyzing against 0.6 M sodium phosphate pH 7.0 at room temperature.

The crystals were fixed in 0.5% glutaraldehyde and rinsed overnight with water before their density was determined by the modified gradient method described by Low and Richards (13). The amino acids L-valine and L-leucine were used as markers.

SDS polyacrylamide gel electrophoresis was performed by the procedure of Weber and Osborn (14) in 12% acrylamide gels. The crystals were heated for 5 min at 100°C in 5% SDS and 2.5% β-mercaptoethanol buffered at pH 7.0 with sodium phosphate. The gels were run at 7 mA/gel for sixteen hours. Ethanol was substituted for methanol in the procedure for staining and destaining the gels. Densitometric scans at 620 nm were run on the Coomassie Blue stained gels.

The trial model for B-phycoerythrin was derived from a modification of our results on C-phycocyanin, where the method is described in more detail (15). This system is considerably simpler since the translation of particles along c is fixed by the space group and we therefore confined our modeling to the hki0 projection. The structure factor for each reflection was calculated by representing each scatterer as a cylinder and using the Fourier transform of a circular hole as a shape function for the projection. The procedure described by Kraut (16) was used to scale the observed and calculated amplitudes and to compute the 1.0-Q and standard R indices.

RESULTS AND DISCUSSION

Crystals of B-phycoerythrin typically grow to 0.2 x 0.2 x 1.0 mm and appear to be suitable for high resolution structural analysis exhibiting diffraction maxima beyond 3Å resolution on oscillation photographs (Fig. 1). Precession photographs of the hk0 and h0ℓ zones are shown in Figure 2. The hk0 zone possesses 6-fold symmetry and the h0ℓ zone only Friedel symmetry. The hk1 zone (not shown) shows only 3-fold symmetry. The crystals, therefore, are assigned to the rhombohedral space group R3 with unit cell dimensions a=111.0Å and α=116.8° or (hexagonal indexing) A=B=189.1Å, C=60.1Å and γ=120°. The volume of the unit cell is 1,860,000Å^3.

Since the space group symmetry requires all three-fold sites to be identical, the B-phycoerythrin aggregate will be limited to a cylinder approximately 109Å in diameter and 60Å in thickness. These limits are consistent with the size of the free particles observed in electron micrographs (6). These dimensions also bear a striking similarity to those reported for C-phycocyanin from A. *quadruplicatum* (17). Although these proteins probably stem from the same ancestral gene, this result was surprising in light of the unusual stoichiometry, $(\alpha\beta)_6\gamma$, reported for B-phycoerythrin (11).

The observed density was 1.211 $\pm$ 0.004 g/cm^3. Assuming a $\bar{v}$=0.74 cm^3/g, this density value implies that 275,000 daltons occupy each three-fold site and would give a V_m=2.26Å^3/dalton. This agrees well with the values of 265 Kdaltons reported by Gantt(7). However, the subunit molecular weights have been reported (7,11) near 17,500 and 30,200 daltons for the light (α and β) and heavy (γ) chains which implies a molecular weight of 240,000 for a particle of $(\alpha\beta)_6\gamma$ stoichiometry. The observed density and this molecular weight would require a $\bar{v}$=0.69 cm^3/g, which is substantially below the values typically expected for proteins (18).

We therefore explored the possibility that we had crystallized an intact $(\alpha\beta)_6\gamma_3$ or $(\alpha\beta)_6\gamma_2$ particle which could be more easily rationalized with both the space group symmetry and density of the crystals. Figure 3 shows a typical result from an SDS gel electrophoresis experiment on the crystalline protein. If we assume that the staining coefficients for the heavy and light chains are nearly equal, approximately ten light chains are found per heavy chain. These results are more consistent with the proposed $(\alpha\beta)_6\gamma$ stoichiometry. The molecular weights for the subunits from the crystalline material were also determined. Approximate values of 28,000 and 18,600 daltons were obtained for the heavy and light chains, respectively (Fig. 3b). With these molecular weights, the agreement between the experimental density and the calculated value based on an $(\alpha\beta)_6\gamma$ particle is

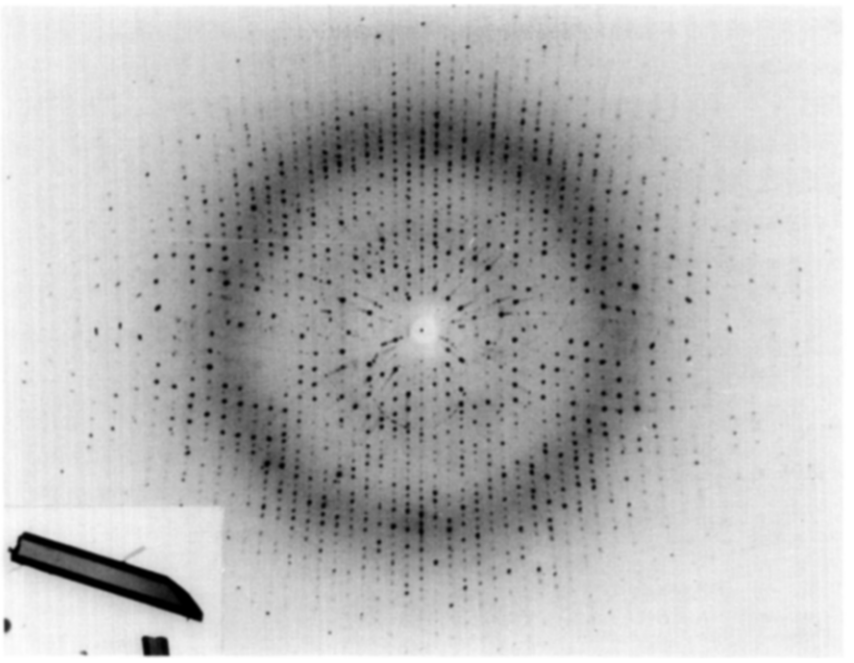

Figure 1. Crystals of B-Phycoerythrin (insert) shown with a 3 1/2° oscillation photograph taken on an Elliott GX6 rotating anode generator with CuK_α radiation. The c axis is along the spindle.

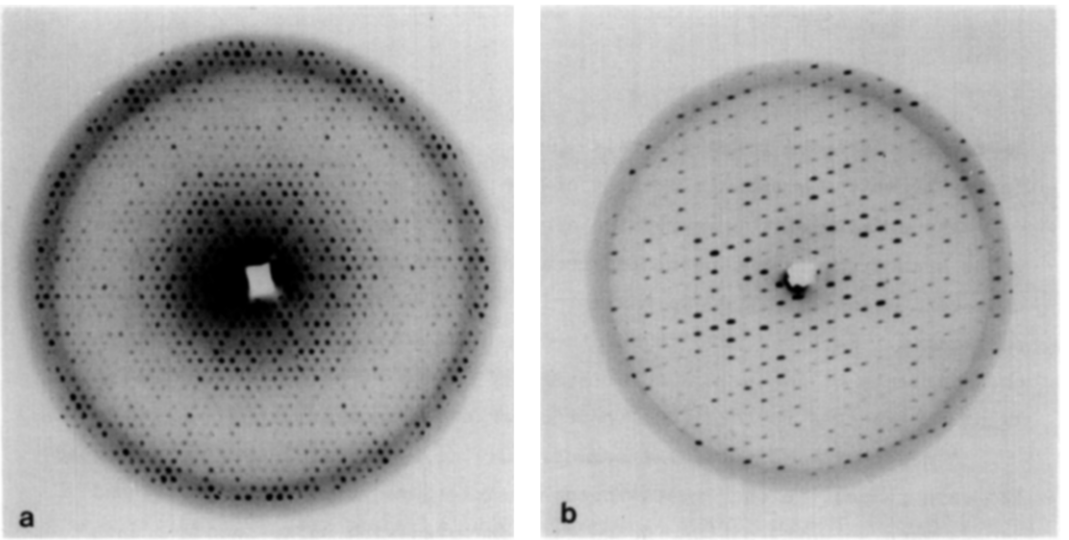

Figure 2. Precession photographs of the hki0 (a) and hoℓ (b) zones of B-phycoerythrin (CuK_α radiation, μ = 10° and 9° respectively). The a^* axis is vertical in both photographs.

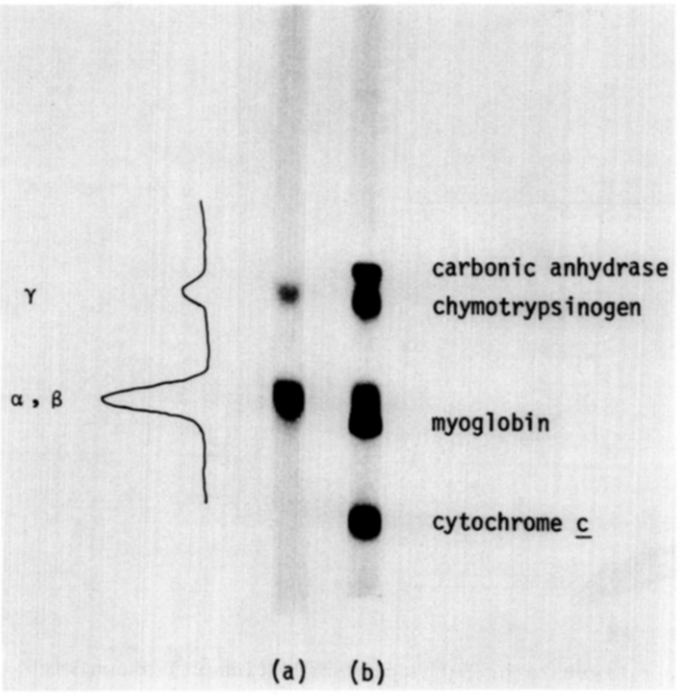

Figure 3. SDS polyacrylamide gel electrophoresis of (a) crystalline B-phycoerythrin with a gel scan indicating the relative amounts of heavy (γ) and light (α and β) chains. In (b) the molecular weights of these chains were checked against marker proteins: cytochrome c (11,700), myoglobin (17,200), chymotrypsinogen (25,700) and carbonic anhydrase (29,000).

improved. In space group R3, this $(\alpha\beta)_6\gamma$ stoichiometry implies disorder for at least the γ subunit portion of the particle.

The packing of the aggregates along the c axis is dictated by space group symmetry with the particles located on the two triads within the unit cell being displaced approximately 20Å ($z = \pm 1/3$) from the one at the origin. Each particle is required to have a three fold axis although the existence of higher molecular symmetry cannot be excluded and, in fact, might be expected for the $(\alpha\beta)_6$ portion of the oligomer.

A model with 32 molecular symmetry having its local 2-fold axes aligned 18° with respect to the crystallographic axes gave values of 1-Q=0.84 and R=0.24 when subunits of 10Å radius were employed with unique nonzero data from 35-20Å resolution. These favorable values are obtained in part because of an unfavorable ratio of unique observations to variables, 11:7, but random models gave considerably poorer values of 1-Q and R, e.g., 1-Q=0.59

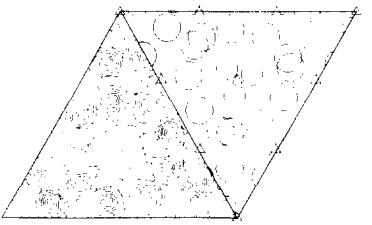

Figure 4. Phases derived from the model shown in the right half of the unit cell were used with the observed amplitudes to produce the electron density projection shown on the left. The model has the mass equivalent of a disordered γ chain around the three-folds surrounded by twelve light chains with local 32 symmetry.

and R=0.63. A Fourier synthesis based on these calculated phases and observed amplitudes gave a projection electron density map that was consistent with the distribution of scatterers proposed in the model (Fig. 4). It is also possible to find reasonable agreement with certain other models, but all of these produced a similar electron density projection when the resulting phases were used with the observed amplitudes. The presence of a local diad oxis slightly rotated from the crystallographic axes also correlates with an intensity spike observed on the hki0 photograph approximately nine degrees from the diagonal of that zone.

This preliminary report on the crystallization of B-phycoerythrin from the red alga *P. cruentum* summarizes the limitations on the size, symmetry and subunit structure of the B-phycoerythrin oligomer that can be extracted from our initial crystallographic data. The results presented here will soon be extended with information available from three dimensional data on the native protein and its heavy atom derivatives.

ACKNOWLEDGMENTS

The authors gratefully acknowledge a gift of B-phycoerythrin from Dr. E. Gantt. We also wish to thank Lynda Lindsay for technical assistance and the Robert A. Welch Foundation and a grant (GM23306-01) from the Public Health Service for financial support.

REFERENCES

1. Wolk, C. P. (1973) Bacteriol. Rev. 37, 32-101.
2. Bogorad, L. (1975) Ann. Rev. Plant Physiol. 26, 369-401.
3. Glazer, A. N. (1976) Photochem. Photobiol. Rev. 1, 71-115.
4. Glazer, A. N., Apell, G.S., Hixson, C.S., Bryant, D.A., Rimon, S., and Brown, D. M. (1976) Proc. Nat. Acad. Sci. U.S.A. 73, 428-431.
5. Vaughan, M. H., Jr. (1964) Ph.D. thesis, Massachusetts Institute of Technology.

6. Gantt, E. (1969) Plant Physiol. 44, 1629-1638.
7. Gantt, E., and Lipschultz, C. A. (1974) Biochemistry 13, 2960-2966.
8. Gantt, E., and Conti, S. F. (1966) J. Cell Biol. 29, 423-434.
9. Gantt, E., and Lipschultz, C. A. (1972) J. Cell Biol. 54, 313-324.
10. Gantt, E., and Lipschultz, C. A. (1973) Biochim. Biophys. Acta 292, 858-861.
11. Glazer, A., and Hixson, C. S. (1977) J. Biol. Chem. 252, 32-42.
12. Zeppezauer, M., Eklund, H., and Zeppezauer, F. (1968) Arch. Biochem. Biophys. 126, 564-573.
13. Low, B.W., and Richards, F.M. (1952) J. Am. Chem. Soc. 74, 1660-1666.
14. Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412.
15. Abad-Zapatero, C., Fox, J.L., Oliver, R. M., and Hackert, M. L. (1977) manuscript in preparation.
16. Kraut, J. (1958) Biochim. Biophys. Acta 30, 265-270.
17. Hackert, M. L., Abad-Zapatero, C., Stevens, S. E. Jr., and Fox, J. L. (1977) J. Mol. Biol. 111, 365-369.
18. Scanlon, W. J. and Eisenberg, D. (1975) J. Mol. Biol. 98, 485-502.