0273-2289/93/4302—0133 $03.00

The Study for Isolation and Purification of R-phycoerythrin from a Red Alga

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Received May 3, 1993; Accepted May 17, 1993

ABSTRACT

An effective procedure for the rapid extraction and purification of the biliprotein R-phycoerythrin from a red alga, *Ceramium isogonum,* was developed. The purified R-phycoerythrin of *C. isogonum* consisted of three components with mol wt 180,000 (6β subunits), 70,000 (6α subunits), and 30,400 (γ subunit), respectively. The phycoerythrin is suitable for use as a natural food coloring and can also be used as a fluorescent label.

Index Entries: R-phycoerythrin; red alga; *Ceramium isogonum.*

INTRODUCTION

Phycoerythrin, a water-soluble phycobiliprotein, is the major light-harvesting pigment of photosystem II in the chloroplasts of red algae. It is packaged in multimeric pigment–protein complexes, called phycobilisomes, located on the stromal surface of the thylakoid membranes. Light energy is absorbed by the phycoerythrin and then transferred to chlorophyll a for photosynthesis (*1,2*). Several types of phycoerythrin exist in algae, and the Ceramiales are reported to contain R-phycoerythrin (*3*).

A distinctive feature of phycobiliproteins is their high fluorescent intensity resulting from high extinction coefficients and quantum yields, and they are therefore widely used as fluorescent tags for molecular probes when covalently linked to antibodies, protein A, biotin, avidin, lectins, and hormones (*4,5*). They can also be used as natural food colorings, and

phycocyanin is already used for this purpose in Japan (*6*). Production of R-phycoerythrin for this purpose requires both a convenient source of algae from which it is to be isolated and a rapid isolation and purification method.

Present extraction procedures have three steps: cell-wall disruption, phosphate-buffer extraction, and separation of cell debris by centrifugation (*7,8*). This is followed by purification steps involving gel, absorption, and ion-exchange chromatography (*9*). Recently, Herara et al. (*10*) have evaluated several extraction and purification methods for phycocyanin from the cyanophyte *Spirulina maxima*, and this article presents the results of an evaluation of a modification of their methods for the extraction of R-phycoerythrin from the marine red alga *Ceramium isogonum*.

MATERIALS AND METHODS

Ceramium isogonum Harvey (Rhodophyta, Ceramiales) grows abundantly in the intertidal zone from where it was collected. The freshly collected algae were cleaned of sand and other contaminants, and air-dried before extraction. For extraction, 15 g (dry wt) algae was washed with 1 m*M* K-phosphate (pH 6.8). The cells were then disrupted and extracted in 1.5% sodium nitrate by freezing (−20°C) and thawing following by passing through an Aminco French Pressure Cell at 80 Ib at 4°C. The extract was centrifuged at 17,000*g* for 60 min at 4°C, and the supernatant treated by adding activated charcoal (2.5 g) and stirring at 4°C for 30 min. The mixture was sequentially filtered through Whatman 541 and 542 and GF/D filters, and concentrated by precipitation by adding solid $(NH_4)_2SO_4$ to give 75% saturation. The precipitate, collected by centrifugation, was then dissolved in 20 mL of 1 m*M* K-phosphate buffer at pH 6.8 and dialyzed against 1 m*M* K-phosphate–0.05*M* NaCl (pH 6.8) at 4°C overnight.

Five milliliters of the dialyzed extract were further purified on a 1.5 × 12 cm DEAE 52 column that had been equilibrated with the 30 m*M* K-phosphate buffer (pH 6.8). The phycoerythrin fractions were eluted with stepwise increasing concentrations of NaCl in 30 m*M* K-phosphate buffer, pH 6.8. The R-phycoerythrin fraction was collected and further concentrated by reprecipitation with 75% saturated ammonium sulfate. The precipitate was collected by centrifugation, resuspended in 1 mL of 30 m*M* K-phosphate buffer (pH 6.8), and concentrated by dialyzation against 30 m*M* K-phosphate buffer–0.05*M* NaCl at 4°C overnight. The amount of protein contained in the dialysate was 0.1 mg/mL. This protein was further identified by polyacrylamide gel electrophoresis. The purity of the phycoerythrin extract was monitored spectrophotometrically by the A_{565}:A_{280} ratio. Protein content was determined by the Coomassie brilliant blue G250 dye binding microassay method (*11*).

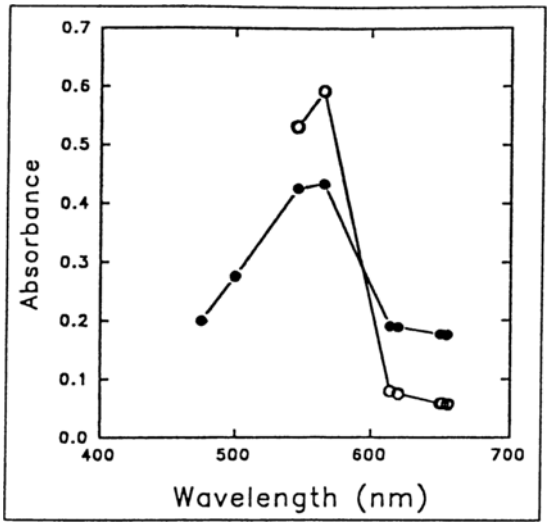

Fig. 1. Absorption spectra of (●) crude and (○) purified R-phycoerythrin extracted from ceramium isogonum.

The R-phycoerythrin was further characterized by discontinuous SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) using a 10.3% separating and 5.2% stacking gel; the acrylamide:*bis*—acylamide solution was 28.736% (w/v) in total. Twenty microliters of the sample were mixed with an equal volume of sample buffer, boiled for 2 min, and loaded into the well of a 150 × 150 × 1.5 mm gel. The conditions for electrophoresis were a constant current of 40 mA for 4 h (*12*). The gel was stained in Coomassie blue G-250, 0. 25% (m/v) methanol:water:acetic acid (3:4:1) and destained in methanol:water:acetic acid (3:4:1) until bands were clearly discernible. The mol-wt standards, 45,000–200,000 (Bio-Rad, Cat No. 161-0303), were run on the same gel in order to estimate the mol wt of the sample.

RESULTS

Extraction by freeze-thawing in 1.5% sodium nitrate effectively extracted the phycoerythrin. Sodium nitrate has been used in preference to the generally used phosphate buffer, since Herrera et al. (*10*) have demonstrated that it have a higher yield and purer product. The extract was further purified by centrifugation and adsorption with activated charcoal to clean it of low-mol-wt proteins. The resulting crude phycoerythrin extract of *Ceramium isogonum* had a maximum absorption at 565 nm characteristic of R-phycoerythrin. The A_{565}:A_{280} ratio for the crude extract was 1.02 (Fig. 1).

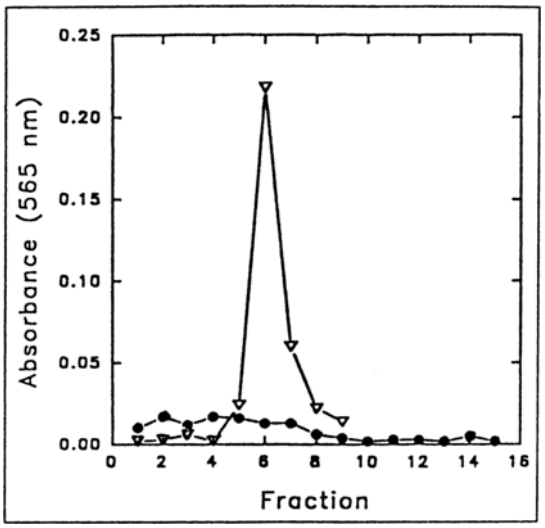

Fig. 2. The absorption of 565 nm of fractions eluted from DEAE 52 column with different concentrations of NaCl (● = 5–250 m*M* NaCl; ▽ = 500 m*M* NaCl in 30 m*M* K-phosphate, pH 6.8).

The crude extract was further purified by ammonium sulfate precipitation. The best purification was obtained with a single ammonium sulfate precipitation step at 75% saturation at 0°C. Stepwise ammonium sulfate precipitation provided no better purification.

The ammonium sulfate fraction was then fractionated on a Sephadex DEAE 52 column. Two milliliters of extract were loaded on a DEAE 52 column (1.5 × 12 cm) and eluted with stepwise concentrations of NaCl in 30 m*M* K-phosphate buffer, pH 6.8. A distinct pink band of phycoerythrin eluted with 500 m*M* NaCl (Fig. 2). The A_{565}:A_{280} ratio of this fraction was 2.02.

The R-phycoerythrin fraction was further concentrated by ultrafiltration with an Amicon filter with a mol-wt cutoff of 10,000 under N_2 at 4°C. This produced 5 mL concentrated R-phycoerythrin with an A_{565}:A_{280} ratio of 2.10. The final concentration of R-phycoerythrin was 115 μg/mL, and the yield of R-phycoerythrin from *C. isogonum* was 0.575 mg from 15 g dry wt algae or 0.383% of algal dry wt. Table 1 summarizes the data from the purification steps, and Fig. 3 gives a flow diagram of the extraction and purification process.

SDS-polyacrylamide electrophoresis of the purified R-phycoerythrin gave three protein bands with mol wt of 30,400, 180,000, and 70,000, respectively. The mol wt of R-phycoerythrin is 240,000. It consists of three subunits (α, β, and γ) with the composition of $\alpha_6\beta_6\gamma$. The mol wt of α, β, and γ subunits determined from their mobility on calibrated SDS-polyacrylamide gels were 11,700, 30,000, and 30,400, respectively.

Table 1
Absorbance Readings of the R-phycoerythrin Extracts during Purification

	Absorbance		
Wavelength	Crude extract	Purified fraction from DEAE 52 column	Concentrated purified R-phycoerythrin
546	0.425		0.530
565	0.433	0.218	0.592
614	0.190		0.079
620	0.188		0.075
650	0.176		0.058
652	0.175		0.056
655	0.175		0.056
280	0.424	0.108	0.282
A_{565}:A_{280} ratio	1.02	2.02	2.10

DISCUSSION

This article reports a simple isolation and purification procedure for R-phycocyanin from the red ceramialean alga *Ceramium isogonum*. Pure R-phycocyanin consisting of the α, β, and γ subunits with mol wt of 11,700, 30,000, and 30,400, respectively, was obtained after extraction by freeze-thawing in 1.5% (w/v) $NaNO_3$ and treatment with activated charcoal followed by precipitation with 75% saturated ammonium sulfate and final purification by Sephadex DEAE 52 column chromatography. The final yield of R-phycoerythrin was 0.38% of algal dry wt.

The method presented can easily be scaled up and, if food-grade phycoerythrin is to be produced, the final chromatography step can be omitted. However, for applications as a fluorescent label, a higher purity product with an A_{565}:A_{280} ratio of >4.0 is required. If the latter product is desired, it may be better to extract in $CaCl_2$ rather than in $NaNO_3$, since Herrera et al. (*10*) have shown that $CaCl_2$ extraction of Spirulina maxima gave a significantly purer product, although the yield was reduced.

This study used the ceramialean red alga *Ceramium isogonum*, which is locally abundant. However, other ceramialean algae should also be suitable as sources of phycoerythrin. Compared to most other red algae, the Ceramiales are much easier to extract and give a higher yield (unpublished results). Since ceramialean algae are abundant on the temperate coasts, they have the potential to become a commercial source of phycoerythrin.

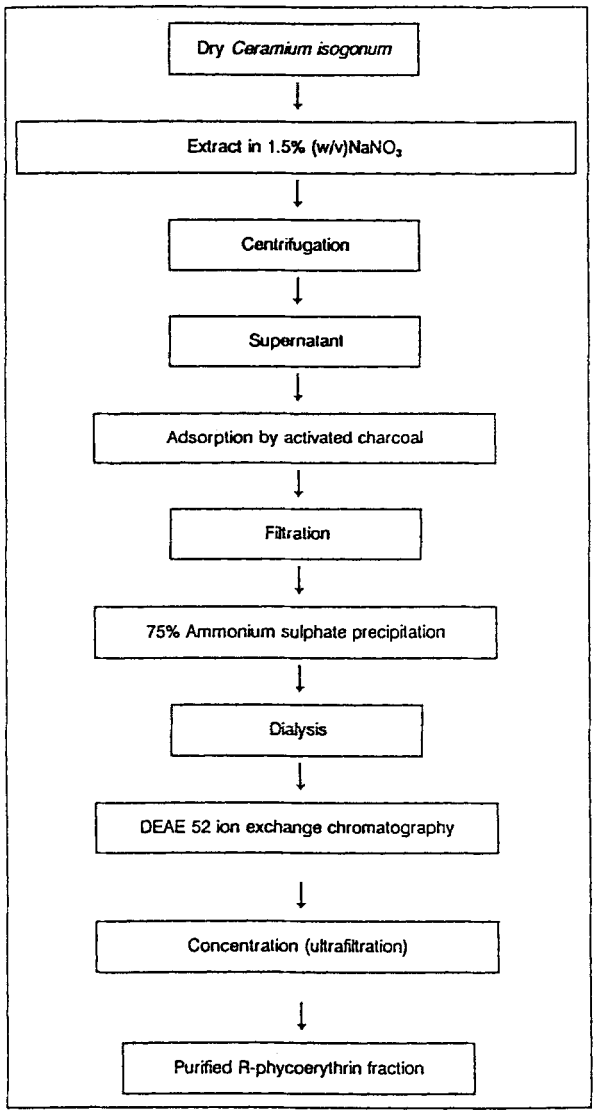

Fig. 3. Flow diagram of the process of extraction and purification of R-phycoerythrin from *Ceramium isogonum*.

Furthermore, many of these algae can be grown in culture, and we have had some success with growing them in air-lift or stirred reactors. The use of cultured algae, rather than field-collected material, has several advantages, including reliability of supply and consistent quality. However, before this can be done on a commercial scale, further work on larger-scale culture methods will be necessary.

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