The Discovery of a Novel R-phycoerythrin from an Antarctic Red Alga*

Robert MacColl†, Leslie E. Eisele, Edwin C. Williams, and Samuel S. Bowser

From the Wadsworth Center, New York State Department of Health, Albany, New York 12201-0509 and the Department of Biomedical Sciences, University at Albany, State University of New York, Albany, New York 12201-0509

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A novel biliprotein, named R-phycoerythrin IV, has been discovered. It absorbs blue light better than any other known red algal biliprotein. The protein was found in Phyllophora antarctica, a benthic macroalga, which grows beneath the coastal waters of McMurdo Sound, Antarctica. Fluorescence emission and fluorescence excitation polarization spectroscopy demonstrated that R-phycoerythrin IV behaved as a typical R-phycoerythrin in the functioning of energy migration and has an emission maximum at 577 nm. The circular dichroism (CD) spectrum of the chromophores was compared with visible absorption spectrum, and both were deconvoluted. This process showed the energy states of various individual chromophores. The molecular weight of the protein suggested a \( \alpha_6 \beta_2 \gamma \) polypeptide structure, and far UV CD studies revealed polypeptides with highly \( \alpha \)-helical secondary structures. Dynamic light scattering indicated that the protein had a 5.54 nm radius, and its shape was nonspherical. R-phycoerythrin was also purified from a second benthic Antarctic red alga, Iridaea cordata. Its spectroscopic properties were similar to those of some R-phycoerythrins from nonpolar regions. The unique spectroscopic properties of R-phycoerythrin IV may help enable the alga to occupy its niche deeper in the water column than the red alga that has the typical R-phycoerythrin.

Photosynthesis is initiated by the harvesting of photons by light-harvesting pigments. The excitation energy migrates to the reaction centers of photosystems I or II where the transition of electron to chemical energy occurs. Biliproteins are photosystem II light-harvesting pigments, which are found in cyanobacteria, red algae, and cryptomonads. With various numbers and types of open-chain, tetrapyrrole chromophores, allophycocyanins, phycocyanins, phycoerythrocyanin, and phycoerythrins are spectrally diverse (1–6).

R-phycoerythrin is isolated from certain red algae with a protein structure of \( \alpha_6 \beta_2 \gamma \). The chromophore content of the \( \alpha \) polypeptide is two phycoerythrobilins, and the \( \beta \) polypeptide contains two phycoerythrobilins and one phycourobilin (7). The % polypeptide has a variable chromophore content (7–9). More than one \( \gamma \) polypeptide is found in an alga (10–12).

Here we report the results of studies on the R-phycoerythrin from an Antarctic alga, Phyllophora antarctica, and show it to be a new form of the protein, type IV. Studies on the purified protein established its size, secondary protein structure, energy-transfer properties, and spectroscopic characteristics. The R-phycoerythrin from a second Antarctic alga, Iridaea cordata, was similar in spectrum to proteins found in nonpolar red algae.

**EXPERIMENTAL PROCEDURES**

P. antarctica and I. cordata were collected at McMurdo Sound at Cape Evans Antarctica. The harvested alga was kept in an aquarium at Antarctica, then frozen at \(-80^\circ\text{C}\) and shipped on dry ice to Albany, NY. The frozen alga was ground repeatedly with a mortar and pestle, followed by extraction into pH 6.0, 0.1 ionic strength, sodium phosphate buffer. Gel filtration with Sepharose 6B yielded two colored bands, the first of which appeared to include some R-phycoerythrin and other pigments. The second band was enriched in R-phycoerythrin and was selected for hydroxyapatite chromatography (Bio-Gel HT, Bio-Rad). A single red band with an \( A_{495}/A_{565} \) of 4.4 eluted with 30 mM NaCl, 40 mM potassium phosphate, pH 7.0. The protein was precipitated with 60% saturated \( \left( \text{NH}_4 \right)_2\text{SO}_4 \) at \( 4^\circ\text{C} \). For further analyses, the protein was harvested, dissolved in pH 6.0, 0.07 M sodium phosphate, and dialyzed against large volumes of that buffer.

The purity of the P. antarctica protein was further established by gel filtration column chromatography using a Waters high performance liquid chromatography system that showed that the major band possessed a spectrum for R-phycoerythrin. The detection system used was a photodiode array detector (13). Dynamic light-scattering measurements (DP-801, Protein Solutions) showed by the polydispersity and base line functions that the R-phycoerythrin was monodisperse and, therefore, pure.

R-phycoerythrin from Gastrodinium coulteri, a red alga not from the Antarctic, was obtained as an ammonium sulfate (60% saturated) precipitate (Schweizerhall, Inc.). It was shown to be pure by an \( A_{495}/A_{280} \) ratio of 4.7 (14).

Absorption spectra were obtained with a DU640 spectrophotometer (Beckman). Samples were maintained at 23 °C using a Peltier device. Protein concentration was based on an absorbivity of 8.2 for a 1 glitter solution at 565 nm (3). The circular dichroism (CD) spectra were recorded on a JASCO J-720 spectropolarimeter as described previously (13). A 1-cm light path was used for CD and absorption measurements in the visible. A light path of 0.5 mm was used in the far UV. Fluorescence emission spectra were obtained using a Perkin-Elmer model LS 50B fluorescence spectrophotometer. Solutions were at \( A_{495} = 0.05 \) or 0.10 in a 1-cm light path in order to minimize the reabsorption of emission. Fluorescence (excitation) polarization was performed as described previously (3). Spectroscopic deconvolution and gel filtration column chromatography was performed as described previously (13).

**RESULTS AND DISCUSSION**

R-phycoerythrin (P. antarctica)—Rennis and Ford (15) studied R-phycoerythrins from many red algae. There are three spectroscopic types, R-phycoerythrins I–III. The R-phycoerythrin isolated from P. antarctica (Fig. 1A) was unique. In particular, its 495 nm absorption band was relatively more intense than that at 565 nm (Table I), an unprecedented observation for the biliproteins of the red algae. The uniquely high 495 nm absorbance was observed in fresh unpurified samples from the earliest time after extraction. The new R-phycoerythrin has been denoted as type IV.

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† To whom correspondence should be addressed: Wadsworth Center, NYSDOH, P. O. Box 509, Albany, NY 12201-0509. Tel.: 518-474-3728; Fax: 518-474-7992; E-mail: robert.maccoll@wadsworth.org.
The first excited state absorption spectra of R-phycoerythrins III and IV. A, type III protein (solid line) was isolated from G. coulteri and the type IV (dots) from P. antarctica. B, R-phycoerythrin from I. cordata.

The absorption bands for this protein were at 565, 545 (shoulder), 495, 372, 307, and 278 nm (data not shown). The bands at 565, 545, and 495 nm are the first excited states of the chromophores. The bands at 372 and 307 nm are higher excited states of the chromophores, and the band at 278 nm is from amino acid residues of the apoprotein. This spectrum was obtained consistently during all stages of the purification and was obtained from several different batches of algae, which argue against any involvement of denaturation. A comparison of the new R-phycoerthrin and a typical type III R-phycoerythrin shows the spectral differences (Fig. 1A). The 565 and 545 nm bands are produced by phycoerythrobilins, and the 495 nm band is primarily from phycourobilin.

The CD spectrum of the chromophores of R-phycoerythrin IV also was distinctive and differed from that of a particular type III protein (Fig. 2). The absorption spectrum could be fitted with a minimum of five components. The CD was also fitted with five components for comparison (Table II) and was in good agreement with the absorption deconvolution. The fits were judged on visual observations of the data and the values of r² (13). The five-component fit indicates that phycoerythrobilins are segregated into three energy states having maxima at 545–558, 529–533, and 568–569 nm. A single bilin may have more than one energy state if the environment of the chromophores differs, or if two or more chromophores are engaged in exciton splitting.

Gel filtration chromatography of the algal extracts showed a molecular weight of 243,000 ± 13,000 for the pigment complex representing 2.7% of the total protein (Fig. 3). The photodiode array detector demonstrated that both bands had the absorption spectrum of R-phycoerythrin. The 243,000 molecular weight complex is typical of all R-phycoerythrins studied thus far and represents an αβ3γ3 subunit structure. The lower molecular weight band probably is a dissociation product from the larger form.

Dynamic light-scattering analyses on R-phycoerythrin IV gave a diffusion coefficient (D_{20,w}) of 3.94 ± 0.13 × 10⁻⁹ cm²/s. The Stokes-Einstein equation was used to calculate a dynamic radius of 5.54 ± 0.18 nm. The frictional coefficient, whereby f = KT/D, was calculated to be 10.45 × 10⁻⁹ g cm⁻¹ s⁻¹. The frictional coefficient of a spherical protein, f₀, having the same molecular weight as R-phycoerythrin IV, is given by:

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f₀ = 6πη(3MV)_{1/3} \quad \text{(Eq. 1)}
\]

where N is Avogadro's number, η is viscosity, M is the molecular weight (260,000), and \( v \) is the partial specific volume of 0.73. The \( f₀ \) was found to be 7.96 × 10⁻⁹ cm⁻¹ s⁻¹, giving a \( f/f₀ \) of 1.31. A ratio of 1.31 suggests that the protein deviates from a spherical shape. This ratio is consistent with the expected disk-like structure of αβ3γ3 complexes, which stack to form the rod portions of the phycobilisomes. The diameters of these disks for various rod-forming biliproteins have been determined from electron microscopy to be in the range of 10.1 to 12.5 nm (3). The diameter of 11.08 nm, determined by dynamic light scattering for R-phycoerythrin IV, fits these dimensions nicely.

CD spectrum of the protein in the far UV showed bands at 193, 210, and 221 nm (Fig. 4). The former is positive and the latter two negative. The spectrum of a completely nonpigmented and about 36,000 molecular weight for a band representing 97.3% of the total protein both pigmented and nonpigmented and about 36,000 molecular weight complex is typical of all R-phycoerythrins studied thus far and represents an αβ3γ3 subunit structure. The lower mo-
The fluorescence emission maximum of R-phycoerythrin IV was at 577 nm, reflecting transfer of energy from high to low energy chromophores (Fig. 5). Excitations at 450, 470, 490, 510, 530, and 550 nm yielded the identical emission spectrum in terms of wavelength maximum and shape. The fluorescence polarization spectrum was obtained (Fig. 5) monitoring emission at 600 nm. Polarization was low at the blue end of the spectrum, and two changes were observed at lower energies. There was a small increase in polarization between 510 and 520 nm and a larger increase between 550 and 570 nm. These changes reflect the occurrences of energy-transfer events and are very similar to the polarization spectrum of type III protein (17).

R-Phycoerythrin (I. cordata)—The purified R-phycoerythrin from I. cordata possessed absorption maxima at 566, 539, and 496 nm (Fig. 1B). The latter band was produced by phycourobilin and the two former bands by different energy states of phycoerythrobilin. The absorption spectrum is clearly different from that of P. antarctica biliprotein and closely resembles a type III protein (Table I). Results for R-phycoerythrin from mesophilic G. coulteri are given for comparison (Table I).

Conclusions—Even at the earliest time after extraction, and before purification had begun, this R-phycoerythrin (P. antarctica) showed its characteristic high 495 nm absorbance. The visible (565 nm) to ultraviolet (280 nm) ratio for the purified protein was as high (4.4) as expected for an R-phycoerythrin, and any loss of 565 nm absorption caused by proteolysis or denaturation of the protein would reduce this ratio. Further evidence that this newly discovered biliprotein was not denatured was that the molecular weight and radius were correct for the expected \( \alpha_6 \beta_6 \gamma \) polypeptide structure, the protein preparation was highly homogenous, the spectrum was stable, the same absorption spectrum was consistently found for the purified protein, and the highly \( \alpha \)-helical secondary structure was characteristic for many biliproteins. Finally, visible absorption studies of intact algae show spectra resembling R-phycoerythrin IV (data not shown). The results support R-phycoerythrin IV from P. antarctica being a new biliprotein. Both the fluorescence emission and fluorescence polarization spectra demonstrate that R-phycoerythrin IV transferred energy very efficiently from high to low energy chromophores as is characteristic of all functioning biliproteins. Both types of fluorescence measurements showed this protein to be very similar to other purified R-phycoerythrins in functionality. The absorption spectrum in the visible region, however, was saliently unique. It is clear that the biliprotein isolated from the Antarctic alga P. antarctica is a new form of R-phycoerythrin.

As visible light enters fresh or marine waters and continues into the water column, it changes in both intensity and color. In general, the light penetrating the ocean surface is primarily blue-green, although various types of water allow differing light transmission. Green light is absorbed better by phycoerythrobilin and blue by phycourobilin. The phycoerythrobilin to phycourobilin ratios vary from species to species (18–22) and the newly discovered R-phycoerythrin IV from Antarctica has an improved faculty to harvest blue light compared with other R-phycoerythrins. Miller and Pearse (23) have surveyed the distribution of red algae at McMurdo Sound. They noted that P. antarctica grew at a greater depth than I. cordata. It is possible
that the novel R-phycoerythrin assists the alga to thrive in these lower light environments.

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