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A NOVEL SUBUNIT SEPARATION PROCEDURE FOR CRYPTOMONAD PHYCOERYTHRIN 545

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SUMMARY: A method to separate phycoerythrin 545, isolated from the cryptomonad alga, Rhodomonas lens, into two subunits has been developed. The method uses no denaturants (urea, guanidine, detergent) but relies on dissociation of the dimeric protein and subsequent aggregation of the β subunit at pH 3.0. The absorption spectra and amino acid composition of the subunits are presented. The spectra of the α subunit was red-shifted relative to β in both pH 3.0 buffer and in acidic 8.0 M urea.

The biliproteins, phycocyanin and phycoerythrin, are found in three types of algae: blue-green, red and cryptomonad. Their most prominent function is as accessory pigments to harvest solar energy and transmit it to the photosynthetic reaction centers. Recent reviews have covered the known physical and chemical properties of this group of proteins (1, 2).

Since they were first discovered in 1969 (3-5), the cryptomonad phycocyanins and phycoerythrins have been compared with the more extensively studied biliproteins from the blue-green and red algae. The most striking difference is that cryptomonad biliproteins (6) apparently do not form the large phycobilisome structures which are so apparent in the electron micrographs of the other algae (7, 8). Some similarities between cryptomonad and other biliproteins have been noted in immunochemical analysis (9) and amino acid sequencing (10). It is also apparent that the energy migration patterns in the cryptomonads are unique (11, 12).

These proteins have an $\alpha_2\beta_2$ polypeptide structure (9, 13) in which both the subunits have chromophores attached. As a step toward better understanding of the structure-function relationships that provide the

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cryptomonad proteins with their energy transfer properties, we have developed a method to separate the α and β subunits of phycoerythrin 545. This relatively simple method is novel in that no denaturants (urea, guanidine, detergent) are used.

MATERIALS AND METHODS

Phycoerythrin 545 was obtained from a unialgal culture of Rhodomonas lens. The alga was grown and the protein purified as described previously (9). Purified protein was exhaustively dialyzed into deionized double-distilled water, freeze-dried, and stored at 4° until needed.

The subunits were separated by sucrose density-gradient centrifugation. The gradients were prepared by layering aliquots of the following solutions: 2.0 or 1.5 ml 65% sucrose, 2.0 ml 50% sucrose, 2.0 ml 35% sucrose, 2.0 ml 27% sucrose, 2.0 ml 20% sucrose, and 0.5 or 1.0 ml sample. All solutions were in pH 3.0 sodium acetate buffer. Centrifuge tubes were cellulose nitrate, and an SW 41 rotor was used in a Beckman Model L3-50 ultracentrifuge, run at 39,000 rpm at 4° for 18-22 h. To obtain evidence for subunit separation some tubes were fractionated into 0.5-ml portions by a Buchler Auto Dense Flow IIC apparatus, and their absorption was measured on a Gilford 2400 spectrophotometer. Otherwise the top band was removed by a pipet and the bottom by puncturing the centrifuge tube. The absorption spectra of the α and β subunits were obtained on a Cary 14 or 118 spectrophotometer.

Sodium dodecyl sulfate gel electrophoresis was performed with pH 6.4 solutions with 12% acrylamide gels.

Amino acid analysis was performed in 6N HCl at 115° as described previously (13). Tryptophan was not determined. At first the α and β subunits were taken off the sucrose gradient and exhaustively dialyzed into double-distilled water. The α subunit (10,000 molecular weight) was mostly lost during this procedure, even when dialysis tubing with a cutoff of 8,000 molecular weight was used; it apparently became tightly bound to the tubing. To avoid this problem the α subunit was dialyzed into 0.01 M ammonium bicarbonate. This salt was mostly volatilized during the freeze-drying step prior to amino acid analysis.

RESULTS AND DISCUSSION

Subunit separation was achieved by adding freeze-dried phycoerythrin 545 (R. lens) to pH 3.0 sodium acetate buffer to obtain a 4 mg/ml solution. Higher protein concentrations generated selected precipitation of the β subunit. The solution was kept at 4° overnight and then centrifuged (48,000 g, 4°, 20 min, on a Sorvall RC-5 centrifuge) to remove all insoluble protein before it was placed on the sucrose gradient.

After the gradient was run overnight, the tubes showed two colored regions (Fig. 1), the slower-sedimenting being blue and the faster red.

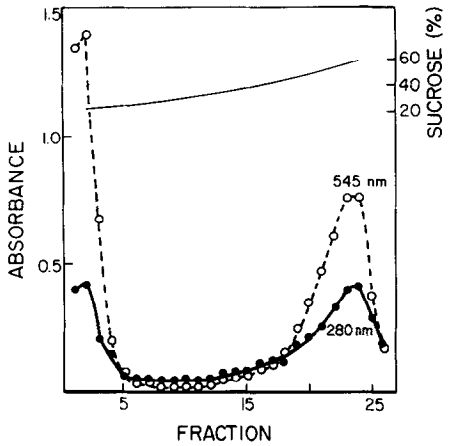

Figure 1: Fractionation of phycoerythrin 545 by sucrose density gradient at pH 3.0.

Electrophoresis showed the faster-sedimenting band to be the β subunit (17,000 molecular weight) and the slower to be the α (10,000 molecular weight). This difference in molecular weight is insufficient to account for the sucrose gradient results. It is therefore obvious that the β subunit undergoes extensive self-aggregation after the $\alpha_2\beta_2$ structure is disrupted by the pH 3.0 conditions.

Both the absorption spectra at pH 3.0 and the amino acid compositions of the two subunits were different. At pH 3.0 the visible absorption spectrum of the α subunit was red-shifted relative to the β (Fig. 2). The λ_{max} was 561 nm for α and 540 nm for β. The ratio of visible to ultraviolet absorption was lower for each subunit (Fig. 2) than for the intact $\alpha_2\beta_2$ protein (9). Hypochromicity of the visible band has likewise been observed for other biliproteins, as for example when C-phycocyanin is denatured by temperature, urea, guanidine hydrochloride, or detergent (14-18). Since our method eliminates the effects of denaturants on the tetrapyrroles, retention of the $\alpha_2\beta_2$ structure seems essential to ensure that the chromophores produce the native spectra.

The absorption spectra of these subunits obtained previously (9) in the presence of sodium dodecyl sulfate showed λ_{max} of 531 (β) and 565 (α).

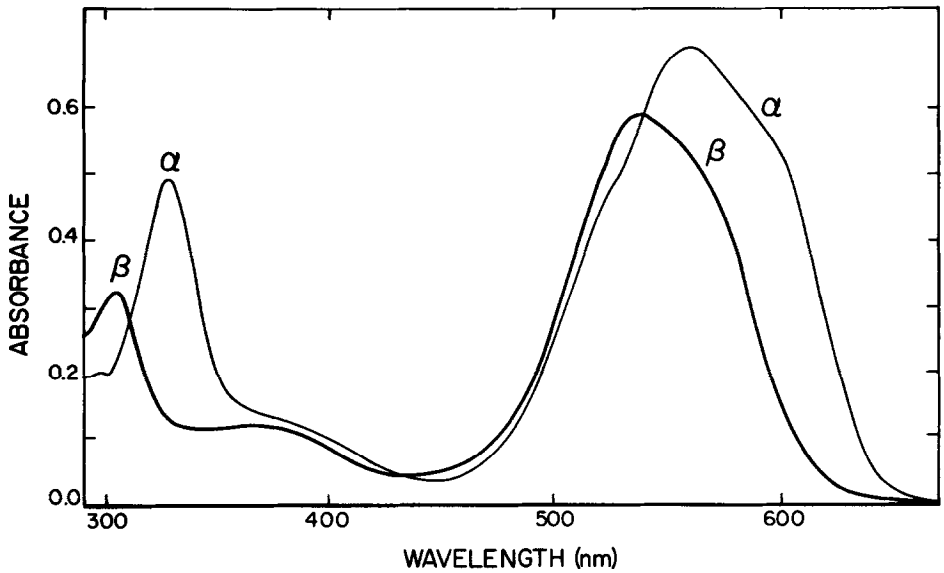

Figure 2: Absorption spectra of α and β subunits of phycoerythrin 545 in pH 3.0 sodium acetate buffer.

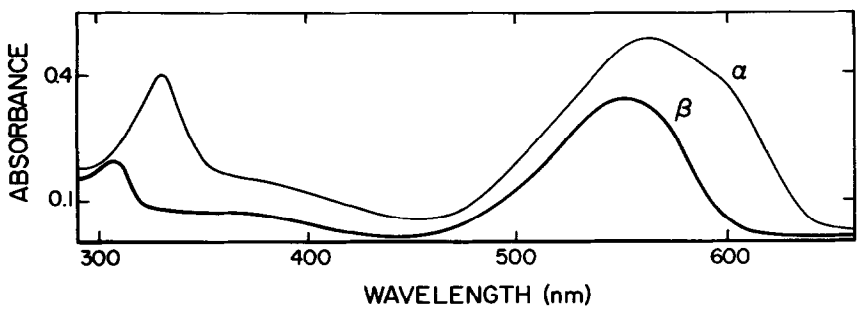

Figure 3: Absorption spectra of α and β subunits of phycoerythrin 545 in pH 3.0, 0.01 M 2-mercaptoethanol, 8.0 M urea. Visible maxima were at 563 nm (α) and 554 nm (β).

Our pH 3.0 spectra, although altered by the disruption of the dimer, are closer to those expected for the native protein: λ_{max} 545 nm and a 561-nm shoulder.

In order to more fully understand the functions of the individual subunits, it is necessary to determine as accurately as possible their spectroscopic properties and then relate them back to those of the dimeric protein. High concentrations of certain denaturants, which were previously used to separate the subunits, are detrimental to such spectroscopic analysis.

TABLE 1

Amino Acid Composition of Phycoerythrin 545 and Its Subunits

(Residues per Mole)

Amino Acid	α[a] (9,000)[c]	β (15,000)	Phycoerythrin 545[b] (24,000)
Asp	8.5	18.2	27.3
Thr	5.7	5.2	11.4
Ser[d]	7.5	21.8	31.0
Glu	9.2	9.3	17.2
Pro	4.0	4.3	7.5
Gly	9.4	12.4	19.7
Ala	11.5	17.9	30.3
Val	5.4	11.0	17.0
Met	2.9	2.3	6.2
Ile	4.1	6.8	10.7
Leu	2.2	12.0	16.3
Tyr	0.9	4.7	6.1
Phe	2.8	3.1	6.3
Lys	8.8	8.2	16.4
His	1.4	trace	1.3
Arg	3.2	5.9	9.2
½-Cys[e]	2.8	6.3	9.3

[a] An unidentified peak eluted in front of lysine for this subunit.

[b] Reference 9.

[c] Molecular weights adjusted for an approximate chromophore content.

[d] Extrapolated to zero hydrolysis time.

[e] Based on hydrolysis to cysteic acid in 6N HCl with dimethylsulfoxide.

Guanidine hydrochloride (6 M), for example, completely bleaches the visible absorption of the α subunit of cryptomonad phycocyanin 645 (13). Our new separation technique thus offers some promise, although with undeniable difficulties, toward future understanding of the structure-function relation-

ship of biliproteins. Separation techniques using bulk denaturants may provide better yields of the subunits since the insolubility problem is avoided.

A solvent of pH 1.9 or 3.0, 0.01 M 2-mercaptoethanol and 8.0 M urea has been reported (19) to eliminate the influence of noncovalent interactions by the polypeptides on chromophore spectra. When the separated α and β subunits of phycoerythrin 545 were dialyzed from pH 3.0 sodium acetate to either of these denaturing solvents, the subunits retained different absorption spectra. Both cryptomonad phycocyanin (20) and cryptomonad phycoerythrin (21) have already been studied in this solvent, and the previous results (21) are in agreement with those of Figure 3. This could possibly suggest that the subunits have chemically different chromophores or that their chemical linkages to the polypeptide chains differ. It has already been suggested (20) that a cryptomonad phycocyanin has three chemically different chromophores. In contrast, blue-green algal phycoerythrin has α and β subunits with identical absorption spectra in 8 M urea (22).

The amino acid compositions of α and β (Table 1) are the first determinations for any cryptomonad phycoerythrin. The α subunit is shown to be richer in threonine, glutamic acid, proline, methionine, lysine, and histidine, and the β subunit has more aspartic acid, serine, leucine, and tyrosine. They are compared to the amino acid composition of the intact protein (9).

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