Subunits of Phycoerythrin from *Fremyella diplosiphon*: Chemical and Immunochemical Characterization†

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ABSTRACT: The α and β subunits of the phycobiliprotein, phycoerythrin, isolated from the filamentous blue-green alga, *Fremyella diplosiphon,* have been separated by chromatography on Bio-Rex 70 ion exchange resin. Analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis shows no detectable cross-contamination of these subunit preparations. The molar extinction coefficients at 552 nm of the α and β subunits in 8 *M* urea are 25,549 and 48,456, respectively. The amino acid compositions of the subunits are very similar. Molecular weights of the α and β subunits are 19,500 and 21,700, respectively, based on the amino acid composition analyses. Antisera prepared against the α subunit reacts with the β subunit, and vice versa. Tryptic peptide maps reveal that the subunits share at least eight common tryptic peptides. These results indicate that the phycoerythrin subunits are chemically very similar.

Cells of the filamentous blue-green alga, *Fremyella diplosiphon,* when grown under fluorescent lamps, synthesize the red pigmented phycobiliprotein, phycoerythrin (Bennett and Bogorad, 1971). Under these conditions, phycoerythrin comprises at least 21% of the total soluble cell protein (Bennett and Bogorad, 1973). Cells grown in red light, however, have no phycoerythrin. The phycobiliproteins phycocyanin and allophycocyanin are present in cells grown with either light source. Action spectra for oxygen evolution and excitation of chlorophyll fluorescence clearly indicate that phycoerythrin and phycocyanin in blue-green and red algae serve to harvest light for photosynthesis, and measurements show that the efficiency of energy transfer for this process approaches 100% (Haxo and Blinks, 1950; Duysens, 1952; Halldal, 1970). Allophycocyanin can serve as an efficient light-harvesting pigment, but its more probable primary role is that of an intermediate in the energy transfer from phycoerythrin and phycocyanin to chlorophyll (Lemasson et al., 1973; Gantt and Lipschultz, 1973). Evidently, the synthesis of phycoerythrin under fluorescent illumination is a physiological response allowing optimal utilization of available light energy for photosynthesis.

The phycobiliproteins apparently exist in vivo in red and blue-green algae as large multimeric aggregates, termed phycobilisomes, which are physically associated with the photosynthetic membranes (Gantt and Conti, 1969; Evans and Allen, 1973). In vitro, the biliproteins also exist as aggregates, the size and extent of aggregation dependent upon pH, ionic strength, and concentration (OhEocha, 1965; Berns, 1971; Eiserling and Glazer, 1974).

Phycoerythrin and phycocyanin isolated from unicellular and filamentous blue-green algal cells each contain two polypeptide subunits of unequal size in a 1:1 stoichiometry (Bennett and Bogorad, 1971; Glazer and Cohen-Bazire, 1971). In both cases, the larger subunit (β) probably contains two bile pigment chromophores covalently bound to apoprotein, and the smaller subunit (α) a single chromophore (Bennett and Bogorad, 1971; Glazer and Fang, 1973). Allophycocyanin has been reported to be comprised of aggregates of either one (Bennett and Bogorad, 1971) or two polypeptides (Glazer and Cohen-Bazire, 1971). The subunits of phycocyanin have been analyzed in detail chemically, physically, and spectroscopically (Berns, 1971; Glazer and Fang, 1973; Glazer et al., 1973). A striking feature revealed by these studies is the similarity between the α and β subunits. The amino acid compositions are very similar, and partial amino acid sequences determined thus far show extensive homology between the two polypeptides (Williams et al., 1974; Troxler et al., 1974).

The present report describes the chemical and immunochemical characterization and comparisons of the subunits of phycoerythrin isolated from *F. diplosiphon.*

Materials and Methods

Cell Growth and Purification of Phycoerythrin. Cells of *F. diplosiphon* (B. and F.) Drouet (strain 481) were cultivated, and phycoerythrin was purified as described by Bennett and Bogorad (1971).

Separation and Isolation of Phycoerythrin Subunits. Phycoerythrin subunits were separated and isolated by a modification of the procedures originally described by Glazer and Fang (1973) for the isolation of phycocyanin subunits. In a typical isolation, 6–8 mg of lyophylized phycoerythrin was dissolved in 5 ml of 8.8% formic acid and 30 m*M* 2-mercaptoethanol and incubated at room temperature in the dark for 14 hr. The solution was applied to a glass column packed with Bio-Rex 70 (Bio-Rad Laboratories, Richmond, Calif.) with bed dimensions of 2.2 × 11.5 cm preequilibrated with 8.8% formic acid (pH 2.2) and 30 m*M* 2-mercaptoethanol. The charged column was washed at room temperature with a 100-ml solution of 2 *M* urea containing 30 m*M* 2-mercaptoethanol and adjusted to pH 2.2 with 5.8 *N* HCl. The subunits were then eluted at room temperature by a stepwise gradient consisting of 40 ml of 4 *M* urea, 60 ml of 6.9 *M* urea, and 40 ml of 9 *M* urea, each containing 30 m*M* 2-mercaptoethanol and adjusted to pH 2.2. Pigmented protein was eluted as a broad peak with the 6.9 *M* urea solution (α subunit) and as a sharp peak near the elution front of the 9 *M* urea solution (β subunit) as

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shown in Figure 1. The peak fractions were pooled, dialyzed against distilled water, and lyophylized.

SDS[1] *Polyacrylamide Gel Electrophoresis.* Purified phycoerythrin and subunit samples were prepared for electrophoresis as follows: 5–20 μg of protein was dissolved in a 40-μl solution containing 0.1 *M* sodium phosphate (pH 7.0), 2% SDS, 2% mercaptoethanol, and 20% glycerol, and heated at 100° for 1 min. Electrophoresis was performed according to the method of Weber and Osborn (1969) using 10% (w/w) gels (0.6 × 10 cm) containing the normal amount of bisacrylamide. The gels were electrophoresed at 7 mA/tube for 5 hr and stained with Coomassie Brilliant Blue according to the method of Fairbanks et al. (1971).

Amino Acid Composition Analyses. Samples containing 500 μg of protein were combined with 2.0 ml of 5.8 *N* HCl containing 0.125% phenol and hydrolyzed in vacuo in glass tubes at 110 ± 2° for 24, 45, and 69 hr. The samples were dried and dissolved in 2.0 ml of 0.2 *N* sodium citrate buffer (pH 2.2) and analyzed on a Beckman Model 120C amino acid analyzer according to the accelerated methods described by Spackman (1967).

Preparation of Antisera and Immunochemical Methods. Full-grown female rabbits were injected in the foot pads with 1–2 mg of either purified undissociated phycoerythrin in normal saline or the separated subunits in 6 *M* urea. The protein solutions (0.5 ml) were mixed thoroughly with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) before injection. Booster injections of 0.5 mg of protein in complete Freund's adjuvant were administered in the foot pads approximately 2 weeks after the initial injections, and the rabbits were bled 2 weeks later. Antisera were prepared as described by Bennett and Bogorad (1973).

Ouchterlony double-diffusion analyses were performed on microscope slides (2.5 × 3.8 cm) coated with 1% Ionagar No. 2 (Oxoid, London) in 0.1 *M* sodium phosphate buffer at pH 7.0. Phycoerythrin and the subunit preparations in 8 *M* urea (2–8 μl) were applied to the outer wells, and antisera (2–8 μl) to the center well, and the slides were incubated 24–48 hr in the dark at room temperature. The developed slides were washed, dried, and stained with Coomassie Brilliant Blue (Clausen, 1969).

Trypsin Digestion and Thin-Layer Peptide Mapping. Phycoerythrin and subunit preparations were alkylated before trypsin digestion by the following procedure. Lyophylized samples (400–750 μg) were dissolved in 0.5 ml of 0.1 *M* Tris-HCl (pH 8.0)–8 *M* urea, and incubated in the dark at room temperature for 5 hr. An equal volume of a solution containing 0.1 *M* Tris-HCl (pH 8.0), 8 *M* urea, and 3.0 mg of iodoacetamide was added, and the mixture was incubated under the same conditions for 45 min. The samples were then dialyzed against 1.5% acetic acid and lyophylized.

Each sample was suspended in 0.4 ml of 0.1 *M* ammonium bicarbonate and 8 μl of a 1 mg/ml solution of trypsin-TPCK (Worthington Biochem. Corp., Freehold, N.J.) in 0.001 *N* HCl was added. The mixture was capped with parafilm and incubated for 4 hr at 25°, an additional 8 μl of the trypsin-TPCK solution was added, and the incubation continued for a further 15 hr. The mixture was then adjusted to pH 3.0 by the addition of 1 *N* HCl and dried at 35° by a stream of nitrogen gas.

Peptide mapping was performed by combined electrophoresis and chromatography in two dimensions on thin-layer sheets of cellulose (Eastman Chromagrams No. 6064, Eastman Co., Rochester, N.Y.). Before use, the cellulose sheets were washed by overnight chromatography at room temperature in chromatographic buffer (1-butanol, pyridine, acetic acid, and distilled water, 90:60:18:72 v/v) in a closed glass chamber (25 × 26.5 × 7 cm) lined with Whatman 3MM paper. The sheets were then dried at 110 ± 2° for 10 min. Each sample was dissolved in 0.04 ml of 0.1 *M* ammonium bicarbonate and carefully applied to one corner of a cellulose sheet. Electrophoresis in the first dimension was performed in a Savant (Hicksville, N.Y.) Model FP-18A water-cooled flat-plate apparatus maintained at 15°. The sheets were prewetted with electrophoresis buffer (pyridine, acetic acid, and distilled water, 1:1:38 v/v, pH 4.5) and electrophoresed at a constant voltage setting of 450 V for 2 hr using Whatman 3MM paper wicks. They were then air dried at 45°. For chromatography in the second dimension, the sheets were immersed in the lined glass chamber containing 100 ml of freshly prepared chromatography buffer. The chamber was preequilibrated at least 6 hr before use. Chromatography was continued for 3 hr at room temperature. The sheets were air-dried and sprayed with a solution containing 1 g of ninhydrin, 700 ml of absolute ethanol, 210 ml of glacial acetic acid, and 29 ml of 2,4,6-collidine. The sheets were air-dried and heated at 110 ± 2° for approximately 10 min, and the developed spots were delineated with a soft lead pencil.

Other Procedures. Absorption spectra were determined on a Cary 14R recording spectrophotometer. Protein levels were measured by the method of Lowry et al. (1951) using cytochrome *c* as standard.

Other Chemicals. Ultrapure urea was obtained from Schwarz/Mann, Orangeburg, N.Y. Acrylamide, bisacrylamide, and SDS were purchased from Bio-Rad Laboratories, Richmond, Calif., and used directly without further purification. Iodoacetamide was obtained from Calbiochem, La Jolla, Calif. All other biochemicals were purchased from Sigma Chemical Co., St. Louis, Mo.

Results

Subunit Stoichiometry. The protein elution profile of the α and β subunits separated on Bio-Rex 70 resin is given in Figure 1. The α subunit eluted with 6.9 *M* urea and the β subunit with 9 *M* urea. Integration of the protein peaks gives a protein stoichiometry of 1:1 which agrees with the relative intensities of the Coomassie Brilliant Blue stained subunit bands separated on sodium dodecyl sulfate gels (Figure 2, and Bennett and Bogorad, 1971).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis of Phycoerythrin Subunits. Examination of sodium dodecyl sulfate gels of the isolated α and β subunit preparations stained with Coomassie Brilliant Blue (Figure 2) showed no detectable cross-comtamination in these preparations. Estimates of the subunit molecular weights based on relative mobilities of proteins of known molecular weights (not shown) gave values of 18,500 ± 10% and 20,550 ± 10% for the α and β subunits, respectively, in agreement with previously reported values (Bennett and Bogorad, 1971; Glazer and Cohen-Bazire, 1971).

Coomassie Brilliant Blue stained gels of the subunit preparations frequently show less intensely stained higher molecular weight bands roughly corresponding to 40,000 and

[1] Abbreviations used are: SDS, sodium dodecyl sulfate; Tris, tris(hydroxylmethyl)aminomethane; TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone.

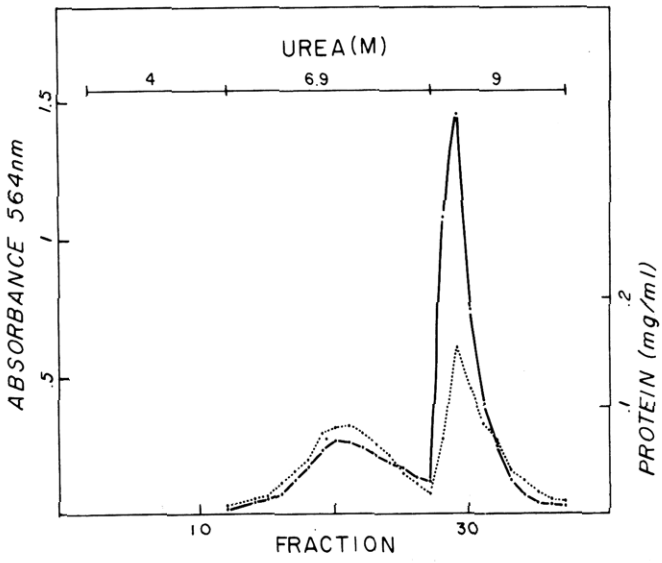

FIGURE 1: Chromatographic elution profile of phycoerythrin subunits on Bio-Rex 70 resin with a stepwise gradient of urea at pH 2.2 and 30 m*M* 2-mercaptoethanol. Absorbance measured at 564 nm (•–•); protein concentration of fractions (mg/ml) (• - - •).

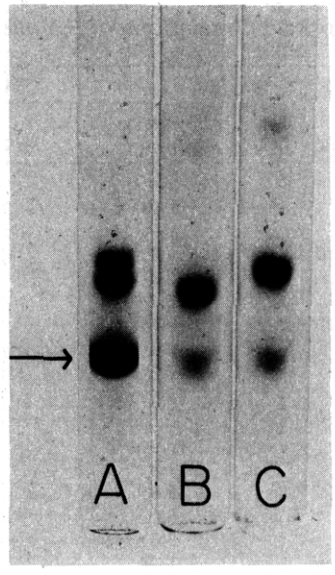

FIGURE 2: SDS polyacrylamide gels of purified phycoerythrin, α and β subunit preparations. Cytochrome *c* was used as a marker (arrow). (A) Purified phycoerythrin, (B) α subunit, (C) β subunit. The gels were stained with Coomassie Brilliant Blue.

80,000. Before staining, these bands are pigmented red, and most likely represent aggregates of subunits. This may be related to the relative insolubility of the isolated subunits observed under certain conditions. For example, both α and β preparations are soluble in 5–10 *M* urea solutions or in acidic buffers below pH 3.0, but insoluble in distilled water or in low ionic strength buffers (for example, 0.05–0.001 sodium phosphate) at neutral pH.

Determination of Subunit Molar Extinction Coefficients. Absorption spectra of the α and β subunit preparations in 8 *M* urea–30 m*M* 2-mercaptoethanol (pH 2.2) were virtually identical with spectra of alkylated phycoerythrin subunits eluted from urea-containing polyacrylamide gels as described by Bennett and Bogorad (1971). Both subunits showed major absorption peaks at 552, 380, and 310 nm.

Molar extinction coefficients at 552 nm ($\epsilon_M^{552\,nm}$) of the α and β subunits in 8 *M* urea–30 m*M* 2-mercaptoethanol (pH 2.2) were determined assuming respective molecular weights of 19,500 and 21,700 obtained from amino acid composition analyses (see below). The $\epsilon_M^{552\,nm}$ for the α subunit was 25,549 and for the β subunit, 48,456. These figures are consistent with the notion that the β subunit contains two chromophores and the α subunit one chromophore per polypeptide chain (Bennett and Bogorad, 1971; Glazer and Fang, 1973).

Amino Acid Composition. The amino acid compositions of the subunits are shown in Table I. The subunits show considerable similarity in amino acid composition. The alanine content is remarkably high, a feature also of the phycocyanin subunits (Glazer and Fang, 1973). Unlike the β subunit of phycocyanin (Glazer and Fang, 1973), the β subunit of phycoerythrin contains at least one histidine residue. Assuming each of the polypeptides contains a single histidine residue and a mean amino residue weight of 110, the molecular weights of the α and β subunits were calculated to be 19,500 and 21,700, respectively. These values correspond well with the molecular weights determined from relative mobilities on sodium dodecyl sulfate gels (see above).

Immunochemical Properties. Antisera prepared against phycoerythrin gave precipitation lines with both α and β subunit preparations on Ouchterlony double diffusion plates (Figure 3). Antisera against α subunit reacts with β subunit preparations and vice versa. Antisera prepared against either of the subunits reacts very weakly with intact phycoerythrin. The precipitation lines observed in all these cases indicated immunochemical identity with no evidence of spurring or crossing-over of precipitation lines.

Tryptic Peptide Maps. Tryptic peptide maps of alkylated phycoerythrin, α and β subunits, showed 18, 12, and 13 detectable ninhydrin-positive spots, respectively (Figure 4). In the case of the α and β subunits, the number of spots detected was less than expected considering the lysine and arginine content of the proteins (α subunit contains 10 arginines + 7 lysines − theoretical number of peptides = 18; β subunit contains 12 arginines + 4 lysines − theoretical number of peptides = 17). This was possibly due to incomplete trypsin digestion, inability to detect peptides weakly reactive with ninhydrin, the occurrence of repeated sequences of amino acids, or overlapping spots.

The α and β subunits appear to share at least eight common tryptic peptides based on similar electrophoretic and chromatographic mobilities on the cellulose sheets. This number represents 67% of the detectable peptides and 44% of the theoretical number of peptides in the case of the α subunit, and 62 and 47%, respectively, in the case of the β subunit. The tryptic peptide map of alkylated undissociated phycoerythrin clearly shows the eight common peptide spots in addition to nine other spots easily attributed in origin to either the α or β subunit proteins. One other peptide spot not detected on the maps of either the α or β subunits appears on the map of undissociated phycoerythrin. The occurrence of this apparently extra peptide remains unexplained. It is conceivable that the accessibility of a specific site for tryptic cleavage differs in the undissociated phycoerythrin.

Before spraying with ninhydrin, pigment was observed in the area coincident with the two closely migrating common peptide spots in the upper right-hand corner of the cellulose sheets (Figure 4). One or both of these peptides may contain the attached bile pigment chromophores.

Table I: Amino Acid Composition of *F. diplosiphon* Phycoerythrin and Its Subunits.[a]

Amino Acid	Mole %			Number of Residues[e]		
	Phycoerythrin	α Subunit	β Subunit	Phycoerythrin	α Subunit	β Subunit
Lysine	2.69	3.73	2.11	10.77	6.61	4.17
Histidine	0.50	0.56	0.51	2.00	1.00	1.00
Arginine	3.93	5.68	6.28	15.74	10.05	12.40
Aspartic acid	8.15	9.44	9.78	32.66	16.72	19.29
Threonine	5.90	5.51	5.18	23.64	9.76	10.22
Serine	8.14	6.27	11.67	32.60	11.10	23.04
Glutamic acid	7.50	7.01	6.84	30.02	12.41	13.51
Proline	4.14	4.33	2.55	16.58	7.67	5.03
Glycine	7.86	10.07	6.72	31.46	17.82	13.28
Alanine	19.92	19.35	19.05	79.74	34.25	37.63
Half-cystine[b]	0.61	n.d.[d]	n.d.	2.45		
Valine	10.15	8.08	11.43	40.64	14.30	22.58
Methionine	1.81	1.37	2.94	7.25	2.43	5.80
Isoleucine	5.99	5.25	3.70	23.98	9.30	7.31
Leucine	6.76	6.16	7.19	27.05	10.90	14.20
Tyrosine	3.38	4.21	2.76	13.52	7.45	5.45
Phenylalanine	1.70	2.98	1.32	6.81	5.27	2.60
Tryptophan[c]	0.85	n.d.	n.d.	3.41		
			Total	400.32	177.04	197.50

[a] The values for threonine and serine were obtained by linear extrapolation to zero time after hydrolysis for 24, 45, and 69 hr. The values for valine, isoleucine, and phenylalanine were taken from the analysis of 69-hr hydrolysates. Values for all other amino acids were taken as averages from analyses of 24-, 45-, and 69-hr hydrolysates. [b] Determined as cysteic acid after performic acid oxidation by the method of Hirs (1967). [c] Determined colorimetrically by the method of Barman and Koshland (1967). [d] Not determined. [e] Determined assuming two histidine residues in phycoerythrin, and one histidine in each of the subunits.

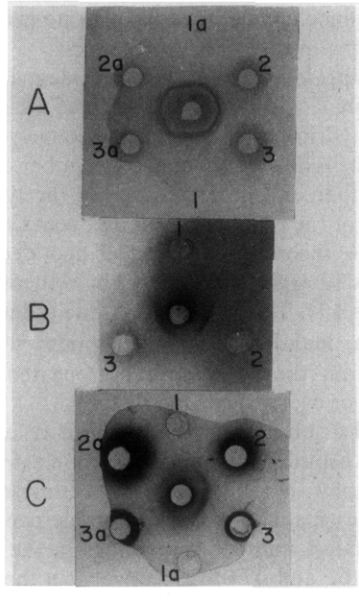

FIGURE 3: Ouchterlony double-diffusion plates of phycoerythrin and its subunits against homologous and heterologous rabbit antisera. Antisera were placed in the center wells as follows: (A) anti-phycoerythrin, (B) anti-α subunit, (C) anti-β subunit. In the outer wells were placed phycoerythrin, 1; α subunit, 2; and β subunit, 3. Samples in wells 1a, 2a, and 3a were phycoerythrin, α subunit, and β subunit, respectively, at ½ dilution. The plates were stained with Coomassie Brilliant Blue.

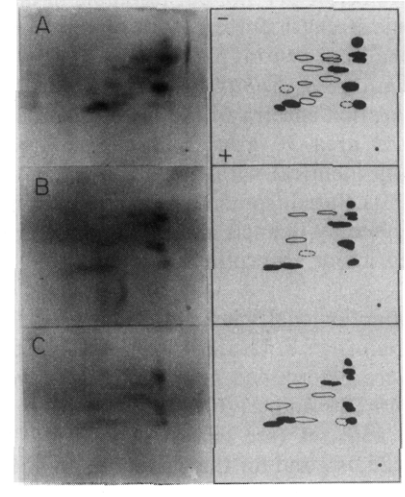

FIGURE 4: Photographs and schematic drawings of tryptic peptide maps of alkylated phycoerythrin, A; alkylated α subunit, B; and alkylated β subunit, C. Common peptide spots are filled in the schematic drawing.

Discussion

The data presented here indicate a close similarity in primary amino acid sequence between large portions of the phycoerythrin α and β subunit polypeptides of *F. diplosiphon*. Preliminary investigations on the primary amino acid sequences of the phycocyanin subunits isolated from the unicellular blue-green alga, *Anacystis nidulans* (Williams et al., 1974) and the eukaryotic alga, *Cyanidium caldarium* (Troxler et al., 1974), likewise point to a close structural homology between the corresponding subunits of this phycobiliprotein. The proposal that the genetic information for the α and β subunits of phycoerythrin and phycocyanin arose through gene duplication in evolution is attractive (Williams et al., 1974), and its strength awaits the further and complete structural analyses of these proteins.

The extensive conservation of subunit sequences during evolution may be attributed to the following: (1) gene duplication may have occurred relatively recently in evolutionary

history, (2) some constraints are imposed by the structure and function of the bile pigment chromophore, or (3) the structural divergence of the subunits may have been restricted by conservation of the aggregate forms of the proteins. In regard to the latter, chromophores must be maintained in high concentrations for efficient energy trapping and transfer. Red and blue-green algae contain large, regularly shaped aggregates of phycobiliproteins (phycobilisomes) located between and in contact with the photosynthetic lamellae. The necessity for such light-harvesting aggregates may limit structural divergence of the component proteins. In this context, it is interesting to consider the phycobiliproteins of the *Cryptomonads* which are not contained in phycobilisomes but are concentrated within the thylakoid vesicles (Gantt et al., 1971). The *Cryptomonad* phycobiliproteins differ chemically and immunologically from those of the red and blue-green algae (MacColl et al., 1973; Glazer et al., 1971a). Therefore, these proteins may have evolved independent of constraints imposed by specific aggregates. Alternatively, the *Cryptomonad* phycobiliproteins may have had a different evolutionary origin.

Also of interest is the structural relationship between the corresponding subunits themselves, for example, the subunits of phycoerythrin and phycocyanin. Such comparisons, for example, may reveal whether a given subunit evolved from a corresponding subunit of a common ancestral biliprotein, or evolved later in time (perhaps by gene duplication) after the acquisition of separate phycocyanin and phycoerythrin genes. Undissociated phycoerythrin does not react immunochemically with antisera prepared against undissociated phycocyanin, and vice versa, even when isolated from the same algal species (Bogorad, 1965; Glazer et al., 1971b). However, there are yet no reports on the immunological relatedness among the subunit moieties of different phycobiliproteins.

The possibility that the α subunit may be a proteolytic breakdown product of the β subunit is not likely. SDS polyacrylamide gel electrophoresis of freshly prepared crude extracts of *F. diplosiphon* cells grown with fluorescent lights clearly show two red-pigmented protein bands corresponding to the phycoerythrin α and β subunits (Bennett and Bogorad, 1971). Specific antibody precipitates of ^{14}C-labeled phycoerythrin from fresh crude extracts of fluorescent light grown cells contain both α and β subunits of phycoerythrin (Bennett and Bogorad, 1973). Finally, when phycoerythrin is purified in the presence of the protease inhibitor, phenylmethanesulfonyl fluoride, the α and β subunits are easily resolved by SDS polyacrylamide gel electrophoresis and detected in roughly equimolar quantities by staining with Coomassie Brilliant Blue (J. Takemoto and L. Bogorad, unpublished observations).

The immunological cross-reactivity between the α and β subunits might be attributed to the antigenicity of the covalently attached phycoerythrin chromophores. There are indications, however, that this is not the case. Antisera prepared against the subunits react weakly or not at all with undissociated phycoerythrin. Antisera prepared against the subunits and undissociated phycoerythrin do not give precipitin lines on Ouchterlony plates with tryptic digests of alkylated phycoerythrin (J. Takemoto and L. Bogorad, unpublished observations). Similarly, Vaughn (1964) showed that tryptic digests of phycoerythrin from *Ceramium rubrum* did not interfere with the immunological reaction between phycoerythrin and homologous or heterologous antisera. A further argument against the possibility of the antigenicity of the chromophore comes from observations of allophycocyanin and phycocyanin. These two phycobiliproteins are immunologically unrelated (Bogorad, 1965; Glazer et al., 1971) although the bile pigment chromophores from each are identical (Chapman et al., 1967; Schram and Kroes, 1971).

The *F. diplosiphon* phycoerythrin subunit structure resembles closely that of phycoerythrin isolated from the unicellular blue-green alga, *Aphanacapsa* sp. (strain 6701) (Glazer and Cohen-Bazire, 1971). However, it differs considerably from the reported subunit structures of phycoerythrins from the eukaryotic red algae, *Porphyridium cruentum* (Gantt and Lipschultz, 1974), which has a B-phycoerythrin containing subunits with molecular weights of 17,300 and 30,000 in a 6:1 molar ratio and a b-phycoerythrin with a single subunit of 17,200 molecular weight, and *C. rubrum* (Vaughn, 1964), which has two subunits with identical molecular weights of 17,000. The phycoerythrins of the red alga *Acrochaetium virgatulum* contain two subunits of molecular weights 19,800 and 22,500 (VanderVelde, 1973) and the two phycoerythrins isolated from a *Cryptomonas* sp. each have two subunits of molecular weights 11,800 and 19,000 (Glazer et al., 1971).

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The Amino Acid Sequence of Ragweed Pollen Allergen Ra5†

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ABSTRACT: The complete amino acid sequence of Ra5, a ragweed pollen allergen, has been determined. Allergen Ra5 is a low molecular weight protein of 45 residues derived from *Ambrosia elatior*, the short ragweed. It contains no detectable carbohydrate or lipid and has four disulfide bridges. The total structure was determined on 1.4 μmol of material and indicates that structural analysis is increasingly possible on relatively small amounts of highly purified material when a combination of automated and manual sequencing techniques and highly sensitive detection systems is employed. This represents the first complete amino acid sequence of a ragweed allergen and it should provide a basis for many structure–function correlative experiments in the field of immediate hypersensitivity.

Almost all wind-pollinated weeds have been implicated in human pollen allergy (Sherman, 1968; Wodehouse, 1971). The most extensively studied of these is the pollen of *Ambrosia elatior*, commonly known as short ragweed. Early work by King *et al.* (1964) suggested that antigen E was the major allergen of ragweed but, recently, several other important allergenically active ragweed pollen allergens have been isolated and characterized (Underdown and Goodfriend, 1969; Griffiths and Brunet, 1971; Lichtenstein *et al.*, 1973; Lapkoff and Goodfriend, 1971, 1974). Allergen Ra5 was of particular interest because of its low molecular weight (5000) and the absence of detectable carbohydrate and lipid. Furthermore Marsh *et al.* (1973a) have shown that IgE-mediated sensitivity to Ra5 was significantly associated with possession of the histocompatibility antigens of the HL-A7 cross-reacting group (Creg). This same association with HL-A7 Creg was reported for formation of human IgG antibodies to Ra5 by Marsh *et al.* (1973b). This represented the first evidence of a strong association between a specific immune response and possession of a specific group of closely related HL-A antigens.

All allergens studied to date have relatively low molecular weights but have otherwise shown no distinguishing chemical features which might correlate with their sensitizing property (Marsh, 1974). However, since relatively little detailed structural information is presently available for these molecules, especially with regard to amino acid sequence, the possibility still exists that some common structural feature, such as a shared sequence stretch, might be associable with the capacity to induce a hypersensitivity reaction. Because of its low molecular weight, its importance in clinical pollenosis, and the relationship between responsiveness to Ra5 and the possession of a group of HL-A antigens, studies were undertaken to determine its complete amino acid sequence. A preliminary account of the findings has been presented (Mole, *et al.*, 1974).

Materials and Methods

Isolation of Ra5. Ra5 was isolated by the method of Lapkoff and Goodfriend (1974). The entire amino acid sequence, including the isolation of the tryptic and chymotrptic peptides, was accomplished on 1.4 μmol of starting material (Figure 1).

Enzyme Digests. Tryptic and chymotryptic digests were prepared from fully reduced and ^{14}C-carboxymethylated Ra5 (O'Donnell *et al.*, 1970) by methods previously described by Press *et al.* (1966). Tryptic digestion was per-

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