Biliprotein Light-Harvesting Strategies, Phycoerythrin 566

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ABSTRACT: A series of experiments on the light-harvesting properties of the cryptomonad biliprotein phycoerythrin 566 has been carried out on purified protein isolated from *Cryptomonas ovata*. Although this pigment has an absorption maximum at 566 nm, a property very close to that of other phycoerythrins, it was found to have a totally unique set of chromophores. The chromophores (bilins) responsible for its absorption spectrum were analyzed by a number of approaches. Chromophore-containing peptides were produced by trypsin treatment and purified in order to isolate the individual peptide-bound bilins free of overlapping absorption. These chromopeptides, after comparison with appropriate controls, showed that three spectrally distinct bilins occurred on the purified oligomeric protein. Two of the bilins were the well-known phycoerythrobilin and cryptoviolin, but the third was previously undiscovered and had an absorption spectrum between that of cryptoviolin and phycocyanobilin. Since the spectral diversity of the three bilins was fully maintained in solvents that minimize the effects of apoprotein on the spectra of the bilins, it is likely that the three bilins are also structurally dissimilar. The α and β subunits, which constitute the protein, were separated by ion-exchange chromatography, and the new bilin was found to be the sole chromophore on the α subunit. It was also found that at least two α subunits could be separated and they both had this unusual bilin (cryptobilin 596). The β subunit, therefore, contained both phycoerythrobilin and cryptoviolin. On the basis of the spectra of the three chromopeptides, the absorption spectrum of the protein was modeled using the known absorptivities of cryptoviolin and phycoerythrobilin. The $\alpha\alpha'\beta_2$ structure, which was determined from molecular weight measurements, was found to most likely contain eight bilins: with two cryptoviolins and one phycoerythrobilin on each β subunit. This was the second biliprotein ever found having three structurally distinct bilins and only the second found with the normally dominant bilin (phycoerythrobilin or phycocyanobilin) occurring in lesser amounts than other bilins. Despite these major structural differences, its absorption maximum at 566 nm is similar to that of C-phycoerythrin (normally observed at 565 nm), which has phycoerythrobilins as its sole bilins.

A major aspect of photosynthesis is the harvesting of solar energy and transmittance of that excitation energy over relatively long distances in very high efficiencies to the two reaction centers. Depending on the ecological niche occupied by the organism, the distribution of solar energy that is available may be quite variable, and therefore a number of pigments are needed with a variety of absorption characteristics. This diversity in antenna pigments is, of course, found in nature, and, for example, land plants may have very different pigments for light harvesting than do marine organisms. In addition, many photosynthetic organisms may vary the quantities and ratios of various pigments, as well as other aspects of their photosynthetic apparatus, in response to fluctuations in available light. It has even been reported that a certain biliprotein may have its bilin content altered in response to light intensity (Yu et al., 1981). There are occasional disputes over whether the intensity or spectrum of light is most important in producing pigment modifications (Saffo, 1987), but, at least for biliprotein-containing organisms, both may perhaps be vital factors (MacColl & Guard-Friar, 1987).

Biliproteins are composed of linear tetrapyrroles that are covalently attached to oligomeric apoproteins. There are generally considered to be three basic groups of biliproteins in terms of absorption spectra: phycoerythrins or phycoerythrocyanin, phycocyanins, and allophycocyanins. There is also extensive spectral diversity within each group. These absorption spectra are constructed from five chromophores (bilins), of which there are two major bilins (phycoerythrobilin and phycocyanobilin) and three minor bilins (cryptoviolin, phycourobilin, and the 697-nm bilin). The minor bilins only occur when one of the major bilins is present. The native spectra are refined via interaction with the apoproteins and bilin–bilin interaction. The properties of these biliproteins and their bilins have been reviewed (Scheer, 1981; Gantt, 1980, 1981; Zilinskas & Greenwald, 1986; MacColl & Guard-Friar, 1987; Zuber, 1985, 1987).

Biliproteins are found in three types of organisms: cyanobacteria (blue-green algae), red algae, and the cryptomonads. Phycoerythrin 566 is a cryptomonad biliprotein isolated from *Cryptomonas ovata*. Previously, our labortory has studied two cryptomonad phycocyanins and have developed models for the flow of excitons through these proteins (MacColl et al., 1988; Csatorday et al., 1988). There is no detailed understanding of exciton migration within any phycoerythrin, and this is a long-term goal of our research. In order to develop these models, it is necessary to begin by understanding the structure of the protein. This work presents the findings of the quaternary structure of phycoerythrin 566, its chromophore content, and the distribution of chromophores on the protein subunits. A knowledge of the chromophores of an antenna pigment is, of course, necessary to understand how it performs its first role of light harvesting.

Experimental Procedures

Phycoerythrin 566 was obtained from harvests of *Cryptomonas ovata* var. *palustris* Prings (UTEX358, The Culture

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Collection of Algae at the University of Texas at Austin) and purified by protocols developed previously for other cryptomonad biliproteins (MacColl & Guard-Friar, 1983). Briefly, these methods included ammonium sulfate (80% saturated) precipitation followed by column chromatography using Sepharose 6B (Pharmacia, Piscataway, NJ) and Ultrogel AcA45 (LKB Instruments, Rockville, MD). Samples with an A_{566}/A_{280} ratio of 5.4 or greater were considered purified. All work on the protein was carried out in sodium phosphate, pH 6.0, 0.1 ionic strength, buffer unless another was specified, and the colum chromatography experiments used in the purification had this buffer plus 0.01% NaN_3. Purified proteins were either stored in a refrigerator under 80% saturated ammonium sulfate or dialyzed into distilled water and lyophilized.

Tryptic chromopeptides were obtained using TPCK-trypsin (Cooper Biomedical) by methods used for another biliprotein (MacColl et al., 1988). The chromopeptides were purified by ion-exchange chromatography using a CM-52 cation-exchange resin (Whatman Limited) and a linear ammonium acetate gradient. The ammonium acetate was at pH 3.5; a 2–200 mM gradient was applied at room temperature. Tryptic chromopeptides were placed in acidic urea after desalting by eluting with 10% acetic acid through a G-50 (Sephadex) gel filtration column and lyophilizing. The α_1, α_2, and β subunits were separated on BioRex 70 cation-exchange resin at pH 2.2 (Bio-Rad Laboratories, Richmond, CA) using a stepwise urea gradient as described previously (Guard-Friar & MacColl, 1986).

Absorption spectra were taken by using a Model 320 spectrophotometer (Perkin-Elmer, Norwalk, CT). High-performance liquid chromatography (HPLC) studies were performed on a Model 342 chromatograph (Beckman) as described previously (MacColl et al., 1988). Elution from the HPLC was monitored by using wavelengths between 550 and 590 nm. These experiments were performed by using a Bakerbond Wide-Bore C18 column and a linear gradient from 0.1% trifluoroacetic acid (TFA) in H_2O to 0.1% TFA/CH_3CN (1:4) at a flow rate of 1.0 mL/min.

The molecular weight of phycoerythrin 566 was obtained by sedimentation equilibrium in pH 6.0 buffer. These experiments were performed at 34 000 rpm, 20 °C, and with a protein concentration of 0.2 mg/mL, using a Model E, analytical ultracentrifuge and absorption optics (Beckman). The subunits of the protein were studied by SDS gel electrophoresis using precast gradient gels (Geltech, Inc., Salem, OH). Gels were usually 3–27% acrylamide.

Other purified biliproteins used in these studies were as follows: C-phycocyanin, cyanobacterium (*Phormidium luridum*); allophycocyanin (*P. luridum*); C-phycoerythrin, cyanobacterium (*Phormidium persicinum*); phycoerythrin 545, cryptomonad (*Rhodomonas lens*); phycocyanin 645, cryptomonad (*Chroomonas* sp.). All these biliproteins were purified by standard methods from organisms grown in this laboratory. R-Phycoerythrin—red alga (*Gastroclonium coulteri*)—was a commercial product (Chemical Dynamics Corp., South Plainfield, NJ).

The α subunit of phycoerythrocyanin was purified by methods devised by Almog and Berns (1984). A mixture of water-soluble proteins was extracted from the cyanobacterium *Anabaena variabilis* and partially purified by ammonium sulfate precipitation (50% saturated). The protein mixture was then dialyzed into H_2O and lyophilized. The proteins (290 mg) were then dissolved in 483 mL of pH 3.0, 0.3 ionic strength, sodium acetate and left overnight at about 4 °C. The solution was then centrifuged at low speed to remove insoluble materials, and the purple supernatant liquid, which was highly enriched in the required subunit, was dialyzed into H_2O and lyophilized. This sodium acetate treatment was repeated 2 additional times to obtain the highly purified α subunit of phycoerythrocyanin.

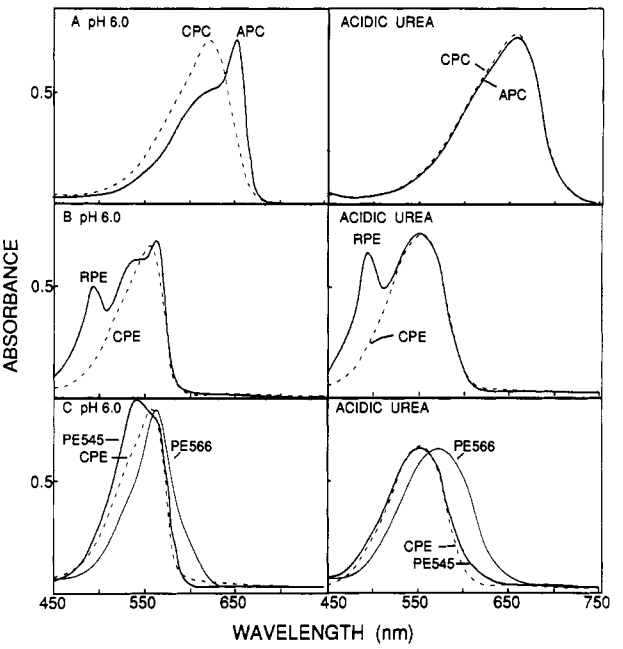

FIGURE 1: Absorption spectra of several biliproteins. In the left column, the proteins are in pH 6.0, 0.1 ionic strength buffer and on the right in 8.0 M urea, pH 2.2: (A) C-phycocyanin (CPC) and allophycocyanin (APC); (B) C-phycoerythrin (CPE) and R-phycoerythrin (RPE); (C) phycoerythrins 545 (PE545) and 566 (PE566) and C-phycoerythrin. In all cases, the absorption spectra are set at about equal absorbances at the particular visible wavelength maxima for comparison purposes.

Results and Discussion

Absorption Spectra of Various Biliproteins. A number of spectrally diverse biliproteins have been studied previously. The comparison of C-phycocyanin and allophycocyanin has provided important insights (Figure 1A), as these biliproteins vary greatly in their absorption spectra (maxima at 615 or 650 nm, respectively) but have been shown to have the identical chromophore, phycocyanobilin. Therefore, when placed in acidic urea—a solvent that minimizes the effect of apoprotein and bilin–bilin interactions on the spectrum—both proteins have identical spectra when normalized at the visible absorption maximum. This result was a clear demonstration that the ability of a bilin to harvest a specific range of solar energy can be highly variable so that only a very select group of bilins is needed to cover the entire spectral region between the Soret and visible bands of chlorophyll *a*.

A comparison of the absorption spectra of C-phycoerythrin and R-phycoerythrin (Figure 1B) was likewise of importance. C-Phycoerythrin had a single maximum here at 557 nm, but 565 nm is commonly reported in the literature, and R-phycoerythrin had three maxima at 563, 541, and 494 nm in pH 6.0 buffer. When the solvent is changed to acidic urea, the 557-, 563-, and 541-nm bands merged at about 555 nm, and the 494-nm band stayed in place. The 494-nm band is caused by phycourobilin while the others are produced by phycoerythrobilin. In acidic urea, the various bands of phycoerythrobilin became spectrally identical. Phycoerythrins can produce variations on their phycoerythrobilins in which the absorbance is restricted to a certain wavelength region having a maximum at either 545 nm (phycoerythrin 545) or 565 nm

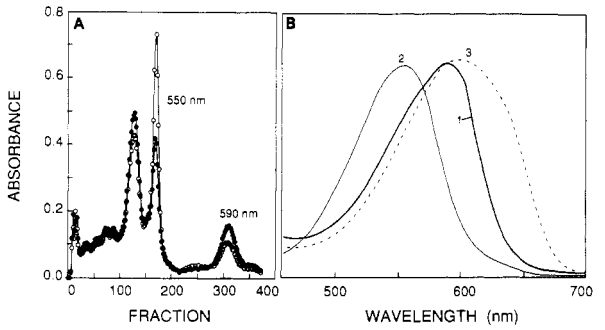

FIGURE 2: Tryptic chromopeptides of phycoerythrin 566. (A) Elution profile of tryptic chromopeptides of phycoerythrin 566 from a CM52 ion-exchange resin; (B) absorption spectra of the tryptic chromopeptides of phycoerythrin 566. These are the peak fractions of the three major bands eluted from the CM52 column. They are in 8 M urea, pH 2.2, and are set at equal maximum absorbance for comparison purposes. Relating them to Figure 2A, they would be (1) fraction 128, (2) fraction 168, and (3) fraction 310.

(C-phycoerythrin), or spread over the entire region (B-phycoerythrin and R-phycoerythrin). The identical story emerged for C-phycoerythrin and phycoerythrin 545 (Figure 1C). Although spectrally quite different in the native state, in acidic urea both proteins have very similar absorption spectra because they are both predominantly composed of phycoerythrobilins. The very small spectral difference in acidic urea at longer wavelengths is caused by the cryptoviolins on phycoerythrin 545. A clear, general picture has emerged in that the variability of absorbance of the major bilins, occasionally combined with smaller quantities from minor bilins, has produced the required spectral diversity for successful biliprotein light harvesting over a wide range of wavelengths.

When a study of phycoerythrin 566 was undertaken, there was no reason to anticipate anything contrary to these concepts. With a 566-nm maximum, phycoerythrin 566 seemed to readily fit the existing pattern since phycoerythrobilin had already been shown capable of absorbing at that wavelength. Phycoerythrins 545 and 566 were both from cryptomonads and were immunochemically nearly identical, and it seemed likely that their spectral differences would be caused by the variability of absorbances in their phycoerythrobilin populations. However, when placed in acidic urea, their spectral differences were totally maintained (Figure 1C). Apparently, although the existing evidence suggested it was unneeded, phycoerythrin 566 has been devised with a saliently different structural answer to a common light-harvesting demand.

Tryptic Chromopeptides. In order to determine what bilins were contributing to the unusual acidic urea spectrum of phycoerythrin 566, the proteolytic enzyme trypsin was used to produce chromopeptides. The mixture resulting from trypsin cleavage was applied to an ion-exchange column, and the elution of chromopeptides was monitored (Figure 2A). Three major bands were obtained, and the absorption spectrum of the peak fraction of each was recorded (Figure 2B). HPLC analysis of these three fractions showed that each contained a single chromopeptide (Figure 3).

Since the bilins were known to be spectrally altered by various chemical treatments (Scheer, 1981; MacColl & Guard-Friar, 1987), possible artifacts from the trypsin digestion and chromopeptide purification had to be considered. Therefore, the α subunit of phycoerythrocyanin (cryptoviolin only) was subjected to identical protocols. The spectrum of its bilin was essentially unchanged by trypsin treatment (Figure 4A). This trypsin-produced chromopeptide gave a single peak when analyzed by HPLC (Figure 5).

As a further control, a cryptomonad biliprotein—phyco-

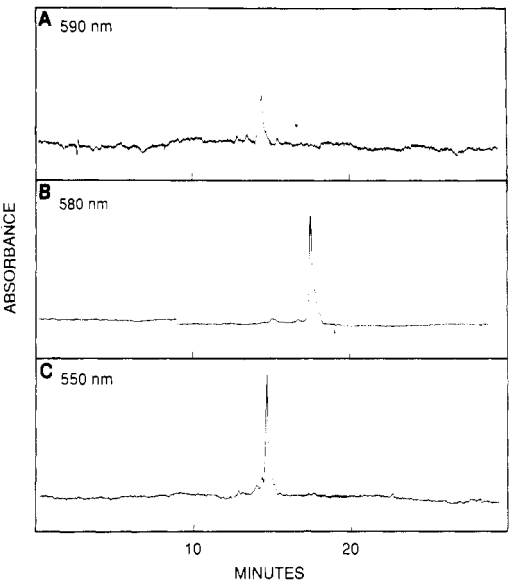

FIGURE 3: HPLC elution patterns for tryptic chromopeptides of phycoerythrin 566. Samples correspond to those shown in Figure 2B and (A) fraction 310, (B) fraction 128, and (C) fraction 168. Their respective elution times are 14.65, 18.19, and 15.02 min.

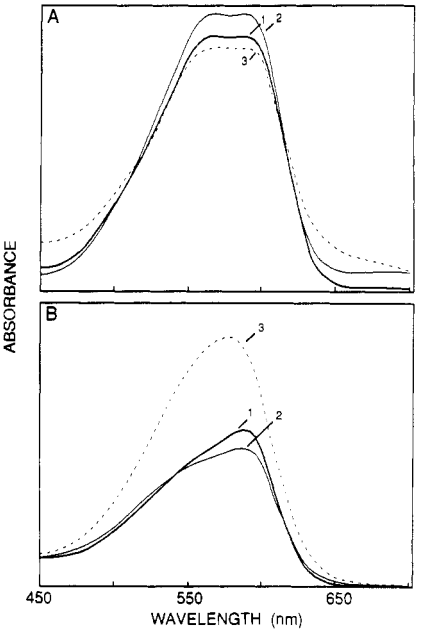

FIGURE 4: Absorption spectra of cryptoviolins. (A) Spectra of authentic cryptoviolins: (1) spectrum before trypsin treatment of the α subunit of phycoerythrocyanin; (2) spectrum after trypsin treatment; (3) spectrum of a chromopeptide from phycocyanin 645. Samples are in 8.0 M urea, pH 2.2. (B) Spectra of earliest eluted tryptic chromopeptides of phycoerythrin 566 from Figure 2: (1) pooling fractions 8–20; (2) pooling fractions 54–88; (3) fraction 128.

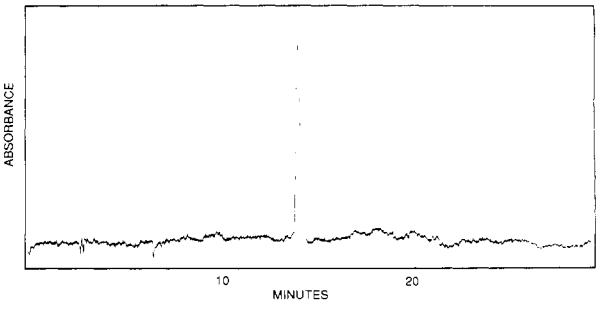

FIGURE 5: HPLC elution pattern for the tryptic chromopeptide of the α subunit of phycoerythrocyanin. Spectrum corresponding to this chromopeptide is shown in Figure 4A.

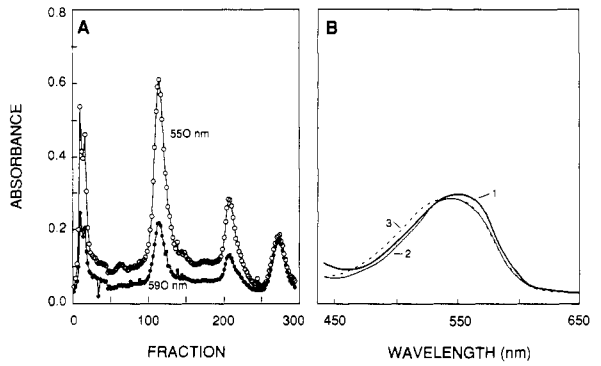

FIGURE 6: Tryptic chromopeptides of phycoerythrin 545. (A) Elution profile and (B) spectra of three phycoerythrobilin-containing chromopeptides. Spectra correspond to the following fractions of (A): (1) fraction 9; (2) fraction 110; (3) fraction 202. Spectra are set to about the same absorbance at the maxima for comparison purposes. These spectra can be compared to the very similar spectra of C-phycoerythrin in acidic urea (Figure 1B) and of the tryptic peptide, number 2, from phycoerythrin 566 (Figure 2B). The band at fraction 266 corresponds in spectrum to a cryptoviolin-like bilin.

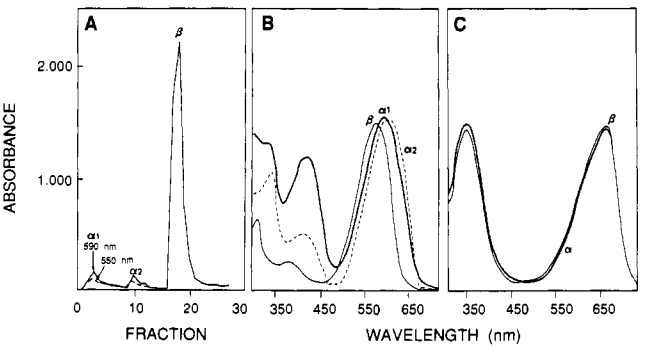

FIGURE 7: Subunits of phycoerythrin 566 and C-phycocyanin. (A) Elution of the subunits of phycoerythrin 566 from an ion-exchange resin in a urea gradient; the two α subunits were eluted in 3 and 5 M urea and the β subunit eluted in 8 M urea; (B) absorption spectra of the subunits of phycoerythrin 566; (C) absorption spectra of the α and β subunits of C-phycocyanin. Samples are in acidic urea. Spectra are set to the same absorbance at their wavelength maxima for comparison purposes.

cyanin 645—was also treated with trypsin and its chromopeptides separated (data not shown). With phycocyanin 645, three elution bands (data not shown) had spectra that corresponded to the bilins of that protein—cryptoviolin (Figure 4A), phycocyanobilin, and the 697-nm bilin. The results showed that the spectrum of cryptoviolin was not altered by the treatment necessary to produce and purify tryptic chromopeptides. Moreover, the spectra (Figure 4B) of the early eluting chromopeptides—first main band and earlier eluting region—from phycoerythrin 566 (Figure 2B) were similar to cryptoviolin from the α subunit of phycoerythrocyanin and the phycocyanin 645 chromopeptide. The spectral heterogeneity among the various chromopeptides of phycoerythrin 566 (Figure 4B) appeared to be in a range acceptable for cryptoviolin. It was, therefore, possible to identify one of the three bilins found on phycoerythrin 566 as cryptoviolin. Cryptoviolin was first demonstrated in work on cryptomonad phycocyanins and later shown to be the sole bilin on the α subunit of phycoerythrocyanin (O'Carra et al., 1980; Bryant et al., 1976; O'hEocha et al., 1964). The structure of cryptoviolin and its photochemistry have been studied by using cyanobacteria as a source (Siebzehnrübl et al., 1989; Bishop et al., 1987). Since the absorption spectra of cryptoviolin from a cryptomonad were shown to be identical with those from cyanobacteria (Figure 4), these bilins should have the same structure.

Phycoerythrin 545 was then used to determine if phycoerythrobilin was present on phycoerythrin 566. Phycoerythrin 545 was selected because it has been previously shown (MacColl et al., 1983) to have a number of phycoerythrobilins and a single cryptoviolin-like bilin. Elution of the tryptic digest showed three phycoerythrobilin-like chromopeptides and a single cryptoviolin-like chromopeptide (Figure 6A). The finding of three phycoerythrobilin-bearing chromopeptides most likely indicated that there were three phycoerythrobilins on the β subunit of the protein. The α subunit had a lone cryptovilin-like bilin. The spectra of each of the three phycoerythrobilin-containing chromopeptides (Figure 6B) were in good agreement with that of authentic, but not trypsin-treated, protein-bound phycoerythrobilin (Figure 1B), and were, also, in good agreement with one of the chromopeptide bands from the tryptic digest of phycoerythrin 566 (Figure 2B, curve 2). Two of the three bilins of phycoerythrin 566 were, therefore, identified.

C-Phycocyanin was also subjected to the trypsin protocol, and its chromopeptides eluted from the same ion-exchange column (data not shown). The resulting absorption spectra were not the same as those exhibited by the third type of chromopeptide of phycoerythrin 566. On the basis of these results with known bilins, it is at least tentatively concluded that this chromopeptide (Figure 2B, curve 3) from phycoerythrin 566 contained a new type of bilin.

Separation of the Subunits. Phycoerythrin 566 was denatured and chromatographed on an ion-exchange column with a urea gradient (Figure 7A). Three bands—two α and one β subunit—were obtained. Multiple α subunits have been found for other cryptomonad biliproteins (Sidler et al., 1985; Guard-Friar & MacColl, 1986; Hiller & Martin, 1987; Martin & Hiller, 1987). The α_1 and α_2 subunits had nearly identical absorption spectra, and these spectra (Figure 7B) were very similar to that of the newly observed bilin (compare to Figure 2B, curve 3). The spectrum of the β subunit appeared to be a combination of phycoerythrobilin and cryptoviolin.

As a control, C-phycocyanin was placed on this same column, and its α and β subunits were eluted with urea in acidic conditions. The spectra of the two subunits (Figure 7C)—both containing phycocyanobilin—were identical with the spectrum of C-phycocyanin under the same solvent conditions (Figure 1A). Since these spectra were red-shifted compared to the α subunits of phycoerythrin 566 (Figure 7B) and were unaffected by the chromatography protocol, this was strong evidence that the α subunit of phycoerythrin 566 did not contain phycocyanobilin.

Sedimentation equilibrium studies of the protein in pH 6.0 buffer gave a molecular weight of 50 800 (data not shown), which supported an $\alpha\alpha'\beta_2$ (or a mixture of $\alpha_2\beta_2$, $\alpha'_2\beta_2$, and $\alpha\alpha'\beta_2$) quaternary structure for the protein, since SDS gels gave the following subunit molecular weights: α, 10 300; α', 11 400; β, 17 300 (data not shown). Both the SDS gel electrophoresis and the urea gradient chromatography results yielded two α subunits. Two α subunits should be considered the minimum number, and additional work will be needed to ascertain if more are present and their roles. The 10 300 and 11 400 molecular weight subunits are called α and α' by previous convention for other biliproteins.

Chromophore Content. In order to calculate the quantity of bilins on phycoerythrin 566, the absorption spectra of the three bilins from the tryptic digest (Figure 2B) were modeled to the absorption spectrum of the protein. In all cases, the spectra were for material in acidic urea. The absorptivities of cryptoviolin and phycoerythrobilin were known (MacColl & Guard-Friar, 1987), but that of the new bilin was not. This

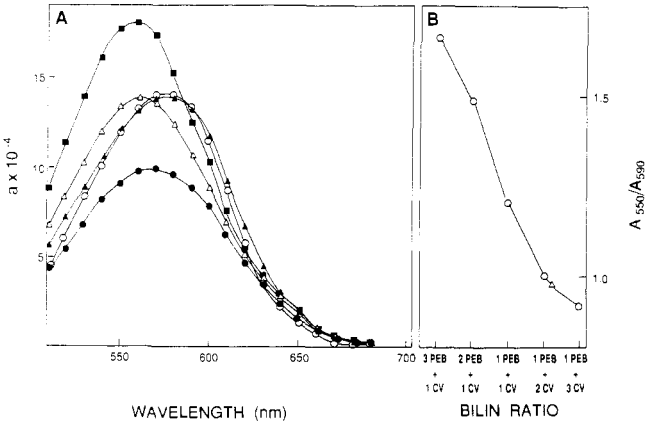

FIGURE 8: Determination of the chromophore content of phycoerythrin 566. Absorbances of phycoerythrin 566 (A) and its β subunit (B) are compared to various combinations of the three spectrally different tryptic chromopeptides (Figure 2B, fraction 128, 168, and 310). For the whole protein (A), the entire spectrum (O) is modeled versus the various combinations [(■) 3 PEB, CV, and CB596; (△) 2 PEB, CV, and CB596; (▲) PEB, 2 CV, and CB596; (●) PEB, CV, and CB596], and for the isolated β subunit (B), the ratio of A_{550}/A_{590} (△) together with possible bilin ratios (O) is given. The bilins are designated as follows: PEB, phycoerythrobilin; CV, cryptoviolin; CB596, cryptobilin 596. All samples are in acidic urea.

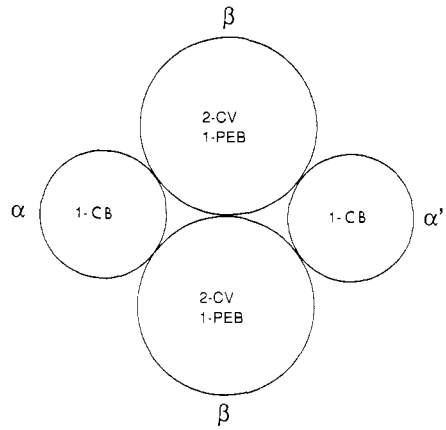

FIGURE 9: Diagram of the subunit and bilin arrangement of phycoerythrin 566. CV is cryptoviolin; PEB is phycoerythrobilin; CB is the newly discovered bilin (cryptobilin 596).

lack of information was overcome since the new bilin absorbed much more strongly at longer wavelengths than the other two bilins, and its absorptivity was assigned so that this low-energy region of the spectrum was correctly fitted. Various combinations of the three bilins were then calculated and compared to the spectrum of the protein (Figure 8A). The absorptivity of the protein was calculated assuming a monomeric ($\alpha\beta$) molecular weight. Attempts using three or five chromophores to fit a monomer were unsuccessful, and four chromophores were clearly the best fit. Furthermore, the combination of two cryptoviolins, one phycoerythrobilin and one new bilin, was best.

To test this finding, the bilin content of the β subunit—obtained from the cation-exchange column (Figure 7A)—was determined (Figure 8B). As predicted from the results for the whole protein (Figure 8A), the β subunit was best fitted by a combination of two cryptoviolins and one phycoerythrobilin. In the elution of the tryptic chromopeptides of phycoerythrin 566 (Figure 2A), there was a broad distribution of fragmented peptides below fraction 100, which had the spectrum of cryptoviolin (Figure 4B). These peptides could possibly represent the partially degraded pieces of a second cryptoviolin chromopeptide. For the $\alpha\alpha'\beta_2$ structure, twice this number of bilins would be needed (Figure 9). Although these procedures yielded excellent fits, they were not a rigorous proof, and confirmatory data are needed especially for the number of cryptoviolins on the β subunit.

Conclusions. The bilin content of phycoerythrin 566 was very unexpected based on predictions from earlier results for a large number of biliproteins. It was the second biliprotein ever shown to have three different bilins—phycocyanin 645 was the other. It was the second biliprotein ever found where a major bilin (phycoerythrobilin) occurred in lower amounts than the normally minor bilins—a particular CU-phycoerythrin from a marine cyanobacterium was the other (Ong et al., 1984). Finally, the protein showed its unusual structure by having a totally new bilin.

The new bilin resembled cryptoviolin somewhat in its absorption. The band maxima were close to each other. Possibly, the new bilin was a form of cryptoviolin, but since the absorption differences persisted in acidic urea, the differences between them could be caused by covalent forces. Perhaps the new bilin was cryptoviolin or phycocyanobilin modified by a different covalent bonding to the apoprotein. It is, also, of note that the α subunit of phycoerythrocyanin, which contains only cryptoviolin, showed very interesting absorption changes after preirradiation (Siebzehnrübl et al., 1989). These authors discussed the possible isomerizations that cryptoviolin may undergo. The observation of this unique bilin by two entirely independent methods—purified tryptic chromopeptides and the separated α subunits—clearly endorsed the validity of the discovery. It was suggested to us that this new bilin be called cryptobilin 596. Likewise, the unusual bilin on the α subunit of phycocyanin 645 would be named cryptobilin 697 for its characteristic absorption in acidic urea.

It was a surprise, at least to us, that phycoerythrin 566 had such an unusual combination of bilins (Figure 9) because its absorption spectrum (Figure 1C) at pH 6.0 did not appear particularly unusual, since a 566-nm maximum could readily be achieved by phycoerythrobilins. Phycoerythrin 545—closely related to phycoerythrin 566—had a native absorption spectrum fairly different from C-phyoerythrin (usual absorption maximum, 565 nm), but its chromophore content of three phycoerythrobilins and one cryptoviolin much more closely resembled the C-phycoerythrin content of five phycoerythrobilins per monomer than did phycoerythrin 566. The light-harvesting strategy of other phycoerythrins was based on the spectral fine tuning of phycoerythrobilins (to have either 545- or 565-nm maxima, or both) with occasional and usually limited help from a minor bilin. Phycoerythrin 566 was designed to fill the same light-harvesting need but through an alternate structural route. Nonetheless, its efficiency in exciton migration was similar to that of other biliproteins, and excitation at 532 nm led to energy transfer in 30 ps (Guard-Friar et al., 1989).

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Registry No. Cryptoviolin, 75026-32-3; phycoerythrobilin, 18097-67-1.

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Purification and Properties of Cystathionine γ-Synthase from Overproducing Strains of *Escherichia coli*†

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ABSTRACT: To characterize the methionine biosynthetic enzyme cystathionine γ-synthase from *Escherichia coli*, we have constructed high copy number plasmids containing the *metB* structural gene but lacking the closely linked *metJ* regulatory gene. When cloned into an appropriate strain, these plasmids can direct the overproduction of cystathionine γ-synthase such that about 10% of the soluble protein is this enzyme. An efficient purification scheme has been developed that has allowed us to obtain gram quantities of enzyme. The active form is a tetramer with subunits of about 40 000 daltons and one pyridoxal phosphate cofactor per monomer. The kinetic constants for several enzyme-catalyzed reactions were determined at 25 °C. The K_m value for the elimination reaction with *O*-succinyl-L-homoserine was calculated to be 0.33 mM with maximal velocity of 460 min^{-1}. The K_m for the elimination (deamination) reaction with vinylglycine was 5.6 mM with maximal velocity of 900 min^{-1}. The K_m values for the replacement reaction were calculated to be 1.0 mM for *O*-succinyl-L-homoserine and 0.05 mM for L-cysteine with maximal velocity of 700 min^{-1}. The enzyme shows an absorption band at 422 nm (ϵ = 8463 M^{-1} cm^{-1}) attributable to the Schiff base form of the pyridoxal phosphate cofactor. Steady-state spectra of reaction complexes show appearance of new longer wavelength absorbing materials during reaction with *O*-succinyl-L-homoserine, vinylglycine, or vinylglycine and L-cysteine. Reaction with *O*-succinyl-L-homoserine and L-cysteine produces only a red shift and slight reduction of the band at 422 nm.

Cystathione γ-synthase (EC 4.2.99.9), the product of the *metB* gene of *Escherichia coli*, catalyzes a γ-replacement reaction to form L-cystathionine and succinate from *O*-succinyl-L-homoserine and L-cysteine (eq 1), or in the absence of cysteine catalyzes a γ-elimination reaction to convert *O*-succinyl-L-homoserine to α-ketobutyrate, ammonia, and succinate (eq 2). The mechanism of the reactions catalyzed by

O-succinyl-L-homoserine + L-cysteine → L-cystathionine + succinate (1)

O-succinyl-L-homoserine + H_2O → α-ketobutyrate + ammonia + succinate (2)

the enzyme from *Salmonella typhimurium* has been the subject of several studies [for example, see Guggenheim and Flavin (1971) and Johnston et al. (1979)], but mechanistic

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