Characterization of the Bilin Attachment Sites in R-Phycoerythrin

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The amino acid sequence around the sites of attachment of all the bilin prosthetic groups of *Gastrochronium coulteri* R-phycoerythrin, (αβ)γγ, have been determined. The sequences of tryptic peptides derived from the α and β subunits are

α-1 PEB Cys*-Tyr-Arg  
α-2 PEB Leu-Cys*-Val-Pro-Arg  
β-1 PEB Met-Ala-Ala-Cys*-Leu-Arg  
β-2 PEB Ile-Asp-Ile-Ala-Gly-Asp-Cys*- (Ser)-Ala-Leu-Ser-Ser-Glu-Val-Ala-Ser-Tyr-Cys-Arg  
β-3 PUB Leu-Asp-Ala-Val-Ala-Ser-Ile-Val-Cys-Ala-Ser-Cys*-Ile-Val-Ser-Ala-Ser-Gly-Met-Ile-Cys*Glub-Asn-Pro-Gly-(Leu)-Ile-Ala-Pro-Gly-Gly-Asn-Cys-Tyr-Thr-Asn-Arg

where the designations α and β refer to the subunits from which the peptides derived. Cysteinyl residues involved in bilin attachment are indicated with an asterisk. Each peptide carries a single bilin, either phycourobilin (PUB) or phycobyrin (PEB).

Spectroscopic studies on the γ subunit indicate the presence of one PEB and three PUB groups. However, five unique tryptic peptides, γ-A through γ-E, were characterized, indicating that *Gastrochronium R-phycoerythrin* is a mixture of at least two species, (αβ)γγ and (αβ)γγ', with γ subunits differing in amino acid sequence. The sequences of the γ subunit bilin peptides (see below) were not homologous to those from α and β subunits of any biliprotein.

γ-A PUB Gly-Thr-Cys*-Tyr-Arg  
γ-B PUB Cys*-Ala-Glu-Thr-Val-Pro-Gln-Ala-Ala-Phe-Glu-Lys  
γ-C PUB Glu-Leu-Tyr-Ala-Ser-(Cys*)-His-His-Glu-(Thr)-Gln-Ile-Phe-Gln-Tyr-Val-Pro-Ala...  
γ-D PUB Ser-Gly-Tyr-Ser-Gly-Ala-Ala-Leu-Asp-Ph-Pro-Val-Ala-Pro-Ser-Leu-Ala-Gly-His-Tyr-Ser-Leu-Thr-Asn-Cys*-Gly-Gln-Ser-Ser-Gly-Ala-(Ser)-(Lys)  
γ-E PUB Leu-(Ser)-Phe-Ala-Leu-Ala-(Cys*)-(Arg)

The bilins in all these peptides are attached through single linkages to a cysteinyl residue, except for the phycourorubin on peptide β-3 which is attached through two thioether linkages to cysteinyl residues 10 amino acids apart.

The availability of small bilin peptides was exploited to obtain more accurate molar extinction coefficients for peptide-linked PEB and PUB groups. Application of these extinction coefficients in the calculation of the bilin content of R-, B-, and C-phycoerythrins shows that there are 5 bilins/αβ in each of these three biliprotein types.

Phycobiliproteins are a family of light-harvesting macromolecules which function as components of the photosynthetic apparatus in cyanobacteria and in two groups of eukaryotic algae, the red algae and the cryptomonads (1, 2). Among these proteins, phycoerythrins are characterized by strong absorption bands in the green region of the spectrum. These red-colored proteins fall into three spectroscopically distinct classes: C-phycoerythrins (λmax ~565 nm), B-phycoerythrins (λmax ~540-560 nm, shoulder ~495 nm), and R-phycoerythrins (λmax ~565, 545, and 495 nm) (3). Only a single type of phycoerythrin is present in any organism.

B- and R-phycoerythrins are proteins of ~240,000 daltons and have a subunit composition (αβ)γγ. All of the subunits bear bilin chromophores (4). The ~550 nm absorbance of phycoerythrin is due to the phycourorubin (PEB)1 chromophore whose structure and modes of linkage to the protein have been the subject of recent detailed studies (5-8). The peak at 495 nm is due to a different bilin, phycourorubin (PUB) (9). The spectroscopic differences between the three classes of phycoerythrins reflect the content and ratio of the PEB and PUB groups (4). A number of attempts were made to determine the structure of PUB, a major prosthetic group in the phycoerythrins of higher red algae, but proof of structure has not yet been achieved nor the mode(s) of linkage to the polypeptide clearly established. Probably the best study on PUB was that of Vaughan (10) who concluded that this bilin was closely related to d-urobilin.

Bilins are linked to the polypeptide chains of the biliproteins through thioether linkages (2). The colored chromophores serve as convenient markers in the purification of bilin peptides. Short chromopeptides provide excellent objects for the determination both of the structure of the bilin and its mode of attachment by nondestructive procedures (5-8, 11).

Only limited information is available on the primary structure of phycoerythrins. Muckel et al. (12) described the amino acid sequences of tryptic PEB peptides from the α and β subunits of C-phycoerythrins. In a comprehensive study, we have recently determined the amino acid sequences and modes of linkage for the five PEB peptides derived from the α and β subunits of a B-phycoerythrin (6) and have provided evi-

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1 The abbreviations used are: PEB, phycobyrin; PUB, phycourorubin; R, R-, and C-PE, R-, and C-phycoerythrins; C-PC, C-phycocyanin; SP-Sephadex, sulfoethyl-Sephadex; TFA, trifluoroacetic acid; P, phosphate; HPLC, high performance liquid chromatography; TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone.
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dence for the structure of both singly and doubly linked PEB groups (5, 7, 8).

This report is concerned with the structure of the chromophores of the R-phycocerythrin of the higher red alga *Gastroclonium coulteri*. We present here amino acid sequences of the two PEB peptides from the α subunit and of two PEB and one PUB peptide from the β subunit. These peptides account for all the bilins on the α and β subunits. Spectroscopic analysis of the γ subunit showed the presence of one PEB and three PUB groups on this polypeptide. However, we isolated five unique γ subunit bilin peptides, one containing PUB and four containing PEB. Consequently, there are at least two γ subunits differing in amino acid sequence in *Gastroclonium* R-phycocerythrin. Further studies, documented in the accompanying paper (13), of small bilin peptides isolated in the course of this work, permitted the unambiguous determination of the structure both singly and doubly linked PUB groups.

**EXPERIMENTAL PROCEDURES AND RESULTS**

The results presented here (Figs. 5 and 7) demonstrate that the α and β subunits of *G. coulteri* R-PE yield five unique trypic bilin peptides. The α subunit carries two PEB groups, each linked to the polypeptide through a single thioether bond. The β subunit carries three bilins: two PEB groups, each linked through a single thioether bond, and a PUB linked through two such bonds. Our earlier study on B-PE produced the unexpected finding that both singly and doubly linked PUB peptides were present within a single protein (6). The present study demonstrates that this situation holds for phycobilins as well.

All five of the α and β subunit bilin peptides show great similarity to the corresponding chromophores derived from *Porphyridium cruentum* B-PE. In fact, three of the peptides from R- and B-PE have identical amino acid sequences (Fig. 9); the two remaining peptides differ in only a few residues (Figs. 9 and 10). These results are remarkable in two respects. The unicellular alga *P. cruentum* belongs to the subclass Bangiophyceae and is a member of the order Rhodymeniales. The close correspondence of the sequences of the B- and R-phycocerythrins, which are chloroplast components, suggests that the chloroplasts of red algae are much more closely related to each other than are the algae within which they are contained. The second noteworthy observation is that in peptides β-3 from B- and R-PE, 37 of the 40 residues are identical (Fig. 10). Yet, the *Gastroclonium* peptide carries a doubly linked PUB whereas the *P. cruentum* peptide possesses a doubly linked PUB. Further, this sequence bears a strong resemblance to residues 38-77 in a region of C-PC which does not contain bilin (Fig. 10). The finding of high sequence conservation over a 40-residue stretch for the β-3 peptides

reinforces the view that the amino acid sequence at the bilin attachment site is not the sole determinant of the bilin identity at that site. Conformational parameters must also play an important role (29).

Spectroscopic analysis of the R-PE γ subunit shows that this polypeptide contains one PEB and three PUB groups. However, five tryptic bilin peptides (Fig. 6), one containing PEB and four containing PUB, assigned to this subunit, were isolated in comparable yield. Yu et al. (30) have shown that *Calithamnion* R-PE contains two γ subunits differing in molecular weight. From studies on the composition of *P. cruentum* phycobilisomes, it appears that B-PE may contain three different γ subunits (31). The present study of the amino acid sequences of bilin peptides derived from the γ subunit indicates that *Gastroclonium* R-PE contains at least two γ subunits, but that these subunits must be very similar in amino acid sequence. It would be more appropriate to describe R-PE as a set of at least two biliproteins differing only in the nature of the γ subunit, i.e. (αβ)γ and (αγ)βγ.

The γ subunit sequences reported here are the first determined for such a subunit. All of the bilins on the γ subunit(s) are attached through single thioether bonds. These peptides comprise ~25% of the total sequence for this polypeptide. In view of the fact that the α and β subunits of all biliproteins show considerable homology (2, 6, 25), it is striking that the γ subunit bilin peptides show no homology to any known α and β subunit sequences. The sole exception is the PEB-containing peptide γ-A which possesses the sequence Cys(bilin)-X-Arg, common to several α and β subunit bilin peptides (Fig. 9).

The structures of both singly and doubly linked PUB chromophores were established by 1H NMR and mass spectrometry analyses of peptides β-3, γ-BV8, and γ-DP (Tables VI

| R-PE     | α-1       | C*-Y-R | α-2       | L*-V-P-R |
|----------|-----------|--------|-----------|----------|
| B-PE     | C*-Y-R    |        | L*-V-P-R  |          |
| R-PE     | β-1       | M-A-A-C*-L*R |        |          |
| B-PE     | M-A-A-C*-L7 |       |          |          |

**Fig. 9. Homologies among bilin attachment sites.** Sequences of *Gastroclonium* R-PE containing peptides α-1, α-2, β-1, and β-2 are compared to the homologous α and β subunit peptides from *P. cruentum* B-PE (6), *Synechococcus* 6301 C-PC (26), and *Fremyella* C-PE (27). The three *Gastroclonium* R-PE-containing peptides which exhibit the Cys-X-Arg pattern at points of PUB attachment are compared in the lower part of the figure. Additional bilin peptide sequences from other biliproteins are tabulated in Refs. 2 and 25. The asterisk indicates the site of bilin attachment.

2 Portions of this paper (including "Experimental Procedures," "Results," Figs. 1-8, and Tables I-XI) are presented in miniprint at the end of this paper. Miniprint is easily readable with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-3364, cite the authors, and include a check or money order for $1.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Fig. 10. Homology between polypeptide regions bearing doubly linked bilins. The homologous peptides from *Gastroclonium* R-PE and *P. cruentum* B-PE, which carry doubly linked PUB and PEB groups, respectively, are compared to each other and to a homologous sequence, not containing bilins, encompassing residues 39-77 of the Synechococcus 6301 C-phycoerythrin β subunit (28). The asterisks indicate the positions of the two bilin-linked cysteine residues and the dots represent identical amino acid residues.

and XI, as detailed in the accompanying report (13).

Previously the molar extinction coefficients for PEB and PUB were determined from absorption spectra in acid urea and amino acid analyses of entire biliproteins as well as of subunits (4, 32). The value reported for PEB was 43.3 mm$^{-1}$ cm$^{-1}$ at 555 nm (32) and for PUB, 104 mm$^{-1}$ cm$^{-1}$ at 495 nm (4). The availability of several pure small PUB- and PEB-containing peptides permitted a much more accurate determination of the molar extinction coefficients applicable to these protein-bound bilins in 8 M urea at pH 3. An ε (550 nm) value of 53.7 mm$^{-1}$ cm$^{-1}$ was obtained for PEB and a value for ε (495 nm) of 94 mm$^{-1}$ cm$^{-1}$ for PUB. The molar extinction coefficient for PUB is identical to that reported for d-urobilin hydrochloride in chloroform (33). Application of the newly determined extinction coefficients to the absorption spectra of B-PE (4) and R-PE in 8 M urea at pH 9 gives chromophore contents in accord with the numbers determined from sequence studies. The new values also lead to a reassessment of the bilin content of C-phycoerythrin. This biliprotein contains only PEB groups. The extinction coefficient of C-phycoerythrin at 555 nm in acid urea is 260 mm$^{-1}$ cm$^{-1}$/αβ (32). The molar extinction coefficient, determined by Glazer and Hixon (32) for protein-bound PEB, of 43.3 mm$^{-1}$ cm$^{-1}$, yielded a value of 6 PEB/αβ. The extinction coefficient of 53.7 mm$^{-1}$ cm$^{-1}$ determined in the present study leads to an estimate of 4.84 PEB/αβ in C-phycoerythrin. It is evident that R-, B-, and C-phycoerythrins all have 5 bilins/αβ monomer.

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Bilin Peptides from R-Phycocyanin

Characterization of the Bilin Attachment Sites in R-Phycocyanin

Bilin peptides were characterized to determine the attachment sites of the bilin chromophores. A thorough investigation of the bilin attachment sites was performed, as depicted in the figure below. The figure illustrates the interaction of bilin peptides with the bilin chromophores. The bilin peptides were characterized to identify the specific attachment sites. The results indicated that the bilin peptides were attached at specific sites on the bilin chromophores. The characterization of the bilin attachment sites provided valuable insights into the structure and function of the bilin chromophores. Further studies are needed to fully understand the role of bilin peptides in the bilin chromophores.

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Bilin Peptides from R-Phycocerythrin

TABLE I

| Amino Acid Composition of Major Bilin-containing Peptide | Subunit 1 | Subunit 2 |
|------------------------------------------------------|----------|----------|
| Asp        | 1.1 (1) | 1.0 (1) |
| Glu        | 1.1 (1) | 1.1 (1) |
| Gln        | 1.1 (1) | 1.1 (1) |
| Ser        | 1.1 (1) | 1.1 (1) |
| Thr        | 1.1 (1) | 1.1 (1) |
| Ile        | 1.1 (1) | 1.1 (1) |
| Val        | 1.1 (1) | 1.1 (1) |

TABLE II

| Acyl-CoA | C3 | C5 | C7 | C9 | C11 | C13 |
|----------|----|----|----|----|-----|-----|
| C3:0     | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |
| C5:0     | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |
| C7:0     | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |
| C9:0     | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |
| C11:0    | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |
| C13:0    | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |

TABLE III

| Acyl-CoA | C3 | C5 | C7 | C9 | C11 | C13 |
|----------|----|----|----|----|-----|-----|
| C3:0     | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |
| C5:0     | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |
| C7:0     | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |
| C9:0     | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |
| C11:0    | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |
| C13:0    | 0.9 (1) | 0.7 (1) | 1.7 (1) | 0.3 (1) |

TABLE IV

| Carboxypeptidase Y Digestion of Peptide | Subunit 1 | Subunit 2 |
|--------------------------------------|----------|----------|
| Asp        | 1.1 (1) | 1.0 (1) |
| Glu        | 1.1 (1) | 1.0 (1) |
| Gln        | 1.1 (1) | 1.0 (1) |
| Ser        | 1.1 (1) | 1.0 (1) |
| Thr        | 1.1 (1) | 1.0 (1) |
| Ile        | 1.1 (1) | 1.0 (1) |
| Val        | 1.1 (1) | 1.0 (1) |

Note: The peptide sequence is the number of residues derived from the sequence determination.

Note: The sequence was determined by gas-phase sequenation and the mass spectrometry (MSP) amino acid derivatives were prepared and analyzed.

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### Table VI

#### Amino Acid Composition of Thermalyl Peptides Derived from the R-Phycoerythrin

| Residue | D, D-P, D-P | D, D-P |
|---------|------------|--------|
| Arg     | 2.67       | 2.48   |
| Asp     | 6.31       | 6.41   |
| Glu     | 6.31       | 6.31   |
| Lys     | 6.07       | 6.07   |
| Phe     | 2.04       | 2.04   |
| Ser     | 2.00       | 2.00   |
| Thr     | 2.00       | 2.00   |
| Trp     | 2.00       | 2.00   |

*Values in parentheses refer to the number of residues obtained from the sequence determination.

*Numbers refer to positions in the sequence of the parent peptides (Fig. 2). (Table VI)

### Table VII

#### Carboxypeptidase Y Digestion of Peptide p-AF

| Residue Released (mol%) | Time (h) |
|-------------------------|----------|
| Arg                     | 4.36     |
| Asp                     | 2.21     |
| Glu                     | 6.31     |
| Lys                     | 6.31     |
| Phe                     | 6.07     |
| Ser                     | 2.00     |
| Thr                     | 2.00     |
| Trp                     | 2.00     |

*Values in parentheses refer to the number of residues obtained from the sequence determination.

*Numbers refer to positions in the sequence of the parent peptides (Fig. 2). (Table VII)

### Table VIII

#### Automated Edman Degradation of R-Phycoerythrin

| Step | Amino Acid | Yield | Final |
|------|------------|-------|-------|
| 1    | 1          | 100   |       |
| 2    | Ala        | 100   |       |
| 3    | Glu        | 92    |       |
| 4    | Trp        | 92    |       |
| 5    | Ser        | 88    |       |
| 6    | Lys        | 88    |       |
| 7    | Glu        | 88    |       |
| 8    | His        | 88    |       |
| 9    | Ser        | 88    |       |
| 10   | Trp        | 88    |       |
| 11   | Lys        | 88    |       |
| 12   | Glu        | 88    |       |

*Values in parentheses refer to the number of residues obtained from the sequence determination.

*Numbers refer to positions in the sequence of the parent peptides (Fig. 2). (Table VIII)
Bilin Peptides from R-Phycoerythrin

Fig. 1. Fractionation of the tryptic digest of RPE. Gel filtration was performed in TBE (40 mm Tris-acetate buffer, 400 mm NaCl, pH 8.0) on a column of Sephadex G-10 (1.5 x 110 cm) and 2.5 ml fractions were collected. The absorbance at 280 mn was correlated for the peptide fraction by HPLC (see "Experimental Procedures"). Fractions I-III were combined as indicated.

Fig. 2. Ion exchange chromatography of Fraction III. MBR-containing peptides α-3, α-6, and α-1 were resolved by chromatographing Fraction III (see Fig. 1) on a 50 x 0.9-cm column pre-equilibrated in 50 mm NaCl, pH 2.1. The column was immediately developed with a linear gradient of 0-60% NaCl in the same buffer at a flow rate of 150 ml/hr and 15 ml fractions were collected. The MBR-containing peptide fractions were combined as indicated.

Fraction Number

Absorbance at 550 nm

550 nm

495 nm

Fraction Number

Absorbance at 549 nm

Fig. 3. CMC-cellulose chromatography of Fraction III. MBR-containing peptide α-3, α-6, and α-1 were resolved by chromatographing Fraction III (see Fig. 1) on a 10 x 0.9-cm column pre-equilibrated in 50 mm NaCl, pH 2.1. The column was immediately developed with a linear gradient of 0-60% NaCl in the same buffer at a flow rate of 150 ml/hr and 15 ml fractions were collected. The MBR-containing peptide fractions were combined as indicated.

Fraction Number

Absorbance

550 nm

495 nm

Fraction Number

Absorbance at 549 nm

Fig. 4. CMC-cellulose chromatography of Fraction I. MBR-containing peptides α-3, α-6, and α-1 were resolved by chromatographing Fraction I (see Fig. 1) on a 10 x 0.9-cm column pre-equilibrated in 50 mm NaCl, pH 2.1. The column was immediately developed with a linear gradient of 0-60% NaCl in the same buffer at a flow rate of 150 ml/hr and 15 ml fractions were collected. The MBR-containing peptide fractions were combined as indicated.

Fig. 5. Summary of the amino acid sequences for the MBR-containing peptides derived from the α and β subunits of Phycoerythrin. The supporting papers and corresponding data are deposited in EMBL-EBI for 2S are presented in Tables I and II. α-Chain residues which were identified by cyanogen bromide digestion (Table II). Free carboxylates were identified as the carboxylated derivatives.

Fig. 6. α-Chains containing peptides. The amino acid sequences of the five positions assigned to α-chain are shown. dM or d10 residues which were confirmed by cyanogen bromide digestion (Table II) of the purified α-chain. dM=10 peptide is shown as Glu-Ala-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Gl
Bilin Peptides from R-Phycocerythin

Fig. 3. Amino acid sequence of the double-linked R-1 bilin peptide. The structures of peptides A-1 and B-10 are displayed. Thrombin
exodigestion of peptide B-1 yielded a double-linked decapeptide, B-27 (residues 17-35 and 27-39), and a monothiol peptide, B-30 (residues
28-35), which were both sequenced by Edman degradation and are denoted by (1); see also Tables V and VI. (+)-Iminosuccinic anhydride
was used for carboxypeptidase Y digestion (Table VIII). Free cysteine was identified as the carboxymethyl derivative.

Fig. 4. Absorbance spectra of the double-linked Phe-containing
peptide R-I in 10 mM Tris-TPA. The single-thiol Phe-containing
peptides R-6 and R-9 had spectra identical to that of R-I.