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A COMPARATIVE STUDY OF THE PHYCOERYTHRIN CHROMOPHORE*

D. J. CHAPMAN, W. J. COLE and H. W. SIEGELMAN

Biology Department, Brookhaven National Laboratory, Upton, New York 11973

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Abstract—Phycoerythrobilin was shown to be the chromophore of all known red biliproteins, the phycoerythrins. The reality of a second chromophore, phycourobilin, in some phycoerythrins is considered. No evidence could be marshalled to support the contention that the so-called phycourobilin is a specific *in vivo* chromophore. Rather it appears to be a protein-phycoerythrobilin complex with absorbance properties similar to a bilene (urobilin). *Chroomonas* phycocyanin contains only the phycocyanobilin chromophore.

INTRODUCTION

THE STRUCTURE of phycoerythrobilin, isolated from C-phycoerythrin was recently elucidated.[1,2] There are several phycoerythrins classified primarily on absorption spectra characteristics. Spectral studies of phycoerythrobilin isolated from some of these phycoerythrins[3] has implicated a common chromophore. We examined all types of phycoerythrins and compared their chromophores with reference phycoerythrobilin prepared from C-phycoerythrin (ex. *Phormidium persicinum*). The phycoerythrobilin chromophores from several phycoerythrins were identical and no indications of structural variations were found. Similar studies on phycocyanobilin,[4] the chromophore of the phycocyanins, also revealed only a single chromophoric group. The only biliprotein examined to date containing more than one bilin pigment is R-phycocyanin.[4] It contains both phycocyanobilin and phycoerythrobilin.

RESULTS

Phycoerythrobilin was the only bilin cleaved from R-, B-, and *Rhodomonas* phycoerythrin. In each case identity with reference phycoerythrobilin (as the dimethyl ester) from C-phycoerythrin was established by visible u.v. absorbance spectra (Table 1), similar i.r. absorbance spectra and chromatographic homogeneity on silica gel with three different solvent systems (carbon tetrachloride:methyl acetate, 2:1 v/v; benzene:ethanol, 8:1 v/v; ethylene dichloride:ethyl acetate, 7:3 v/v).

Only one bilin pigment was recovered from the phycocyanin of *Chroomonas*. Identity of the pigment with reference phycocyanobilin (as the dimethyl ester) from C-phycocyanin was established by similar absorbance spectra in the visible u.v. (Table 1) and i.r. region and chromatographic homogeneity in the above three solvent systems.

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[1] D. J. CHAPMAN, W. J. COLE and H. W. SIEGELMAN, *J. Am. Chem. Soc.* **89**, 5976 (1967).
[2] D. J. CHAPMAN, W. J. COLE and H. W. SIEGELMAN, in preparation.
[3] P. Ó CARRA, C. Ó hEOCHA and D. M. CARROLL, *Biochem.* **3**, 1343 (1964).
[4] D. J. CHAPMAN, W. J. COLE and H. W. SIEGELMAN, *Biochem. J.* **105**, 903 (1967).

TABLE 1. ABSORBANCE MAXIMA (nm) OF PHYCOERYTHROBILIN AND PHYCOCYANOBILIN DIMETHYL ESTERS PREPARED FROM PHYCOERYTHRINS AND PHYCOCYANINS

Source of bilin dimethyl ester	Solvent				
	5% HCl-methanol		Saturated zinc acetate in ethanol		
	Absorption maxima (nm)				
Reference C-phycoerythrobilin	591	326	603	557	336
B-phycoerythrin	590	326	603	557	336
R-phycoerythrin	591	327	603	557	335
Rhodomonas phycoerythrin	591	326	603	556	336
Reference phycocyanobilin	687	375	664		375
Chroomonas phycocyanin	685	373	663		375

DISCUSSION

R-phycoerythrin and B-phycoerythrin both show a distinct absorbance maximum or shoulder respectively at 498–500 nm.[5] This maximum has been attributed to a third chromophore, phycourobilin, since the bilene urobilins all show distinct absorbance maxima in this region.

Chromopeptides[5,6] and subunits (P-chloromercuribenzoate-induced dissociation)[7,8] have been prepared from R- and B-phycoerythrin. In many instances these show very prominent 500 nm absorbance maxima. The failure to eliminate this maximum, even from small fragments or subunits is the mainstay of the argument for phycourobilin. In the present work attempts to isolate this chromophore have been unsuccessful. No urobilin was liberated by methanol hydrolysis or by Nagarse, a proteinase, that liberates phycoerythrobilin and phycocyanobilin.[9] Chromatographic examination of the *Aplysia* defensive pigment, aplysioviolin, derived from R-phycoerythrin revealed no urobilin zone (unpublished observations). The urobilin[5] liberated by the silver sulphate method[10] probably arises from the action of acetic acid on the acetic acid-liberated phycoerythrobilin. Phycoerythrobilin is unstable and readily isomerizes to a urobilin.[3,11]

There is additional circumstantial evidence that the 500 nm absorbance maximum is the result of protein-phycoerythrobilin interaction. The absorbance spectra of R-phycoerythrin dissolved in 5 M guanidine-HCl, both before and after methanol hydrolysis are nearly identical (Fig. 1). If two chromophores were present, the ratio $E_{500\ nm}:E_{560\ nm}$ would be expected to increase noticeably, reflecting the approximately 20 per cent liberation of phycoerythrobilin (560 nm peak) only. The ratio is virtually unchanged (0·94 before; 0·99 after) suggesting that both maxima result from the same chromophore (phycoerythrobilin).

The absorbance spectra of the phycoerythrins are markedly pH dependent. The absorbance spectrum of R-phycoerythrin at pH 10 has a predominant broad 500 nm absorbance

[5] C. Ó hEOCHA, *Chemistry and Biochemistry of Plant Pigments* (edited by T. W. GOODWIN), p. 175, Academic Press, London (1965).
[6] T. FUJIWARA, *J. Biochem.* (*Tokyo*) **48**, 317 (1960).
[7] E. FUJIMORI and J. PECCI, *Arch. Biochem. Biophys.* **118**, 448 (1967).
[8] J. PECCI and E. FUJIMORI, *Biochim. Biophys. Acta* **137**, 147 (1967).
[9] H. W. SIEGELMAN, D. J. CHAPMAN and W. J. COLE, *Arch. Biochem. Biophys.* **122**, 261 (1967).
[10] A. PAUL, *Acta. Chem. Scand.* **4**, 239 (1950).
[11] W. J. COLE, C. Ó hEOCHA, A. MOSCOWITZ and W. R. KRUEGER, *European J. Biochem.* **3**, 202 (1967).

maximum[12] with a subsidiary 560 nm maximum. A 500 nm absorbance maximum is induced in C-phycoerythrin at pH 10, a biliprotein that apparently has only a phycoerythrobilin chromophore. The absorbance spectra of C-, B- and R-phycoerythrin are all very similar at pH 10[12] (and unpublished observations of this laboratory). Denatured C- and R-phycoerythrins dissolved in 5 M guanidine-HCl (acid) and in 2 M Tris (tris (hydroxymethyl) aminomethane) in 5 M guanidine-HCl (alkaline) (Fig. 2) have predominant 515 and 510 nm absorbance maximum, respectively. In 5 M guanidine-HCl, C- and R-phycoerythrins show absorbance maxima at 560 nm, and 560 and 500 nm respectively. Under the alkaline conditions the 515 nm absorbance maximum predominates and appears in C-phycoerythrin (Fig. 2).

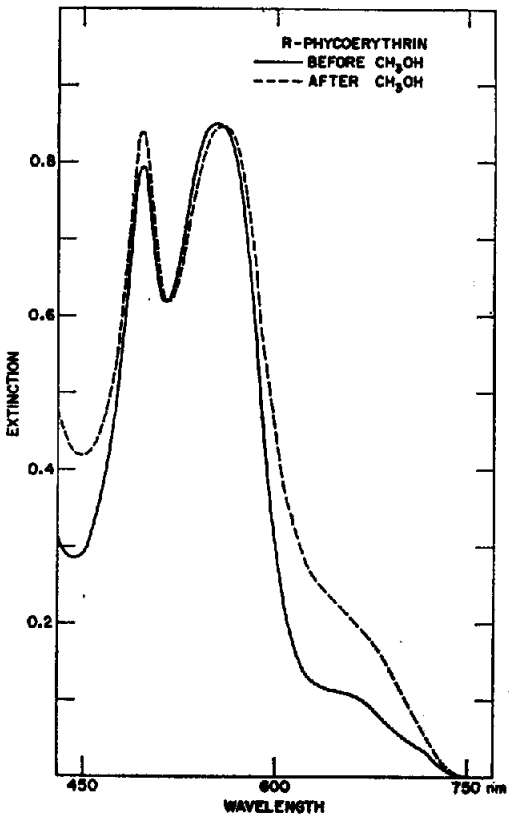

FIG. 1. ABSORBANCE SPECTRA OF R-PHYCOERYTHRIN IN 5 M GUANIDINE-HCl BEFORE (——) AND AFTER (- - - -) METHANOL HYDROLYSIS.

The fluorescence spectrum of R-phycoerythrin is suggestive of only one chromophore. Irrespective of the wavelength of excitation,[12] the fluorescence emission spectrum was reported to have only one maximum at 578 nm, corresponding to phycoerythrobilin. R-phycocyanin, which contains phycocyanobilin and phycoerythrobilin[4] was reported to have two maxima in the fluorescence emission spectrum, corresponding to the two chromophores.[5]

Chroomonas-phycocyanin further illustrates the complexity of the protein-bilin interactions. Ó hEocha *et al.*[13] have suggested that the lower maximum (585 nm) is due to a second

[12] M. VAUGHAN, Ph.D. Thesis, Massachusetts Institute of Technology (1965).
[13] C. Ó hEOCHA, P. Ó CARRA and D. MITCHELL, *Proc. R. Irish Acad.* 63B, 191 (1964).

chromophore, the other (650 nm) being due to phycocyanobilin. Only phycocyanobilin was cleaved from this phycocyanin, and there was no indication of a second chromophore. Denatured *Chroomonas*-phycocyanin dissolved in 5 M acetic acid or in 5 M guanidine-HCl has a single absorbance maximum at 678–688 nm both before and after methanol hydrolysis (cleavage of the chromophore), implying a single chromophore. The single maximum (660 nm) in the fluorescence emission spectrum also suggests a single chromophore.

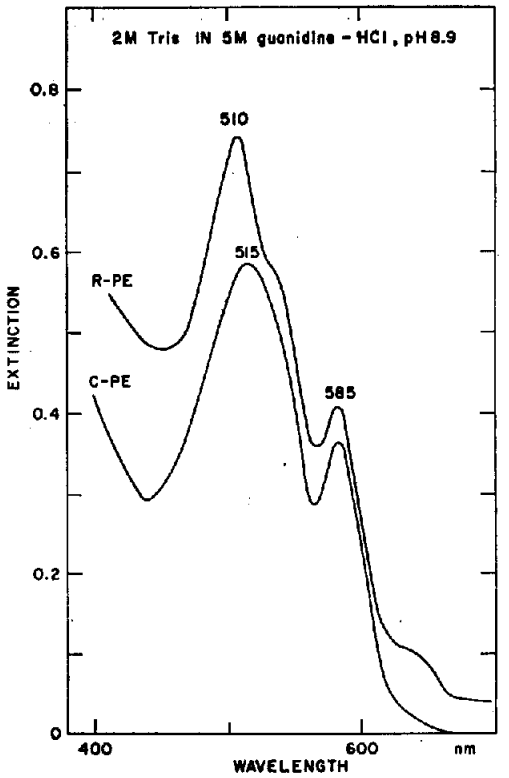

FIG. 2. ABSORPTION SPECTRA OF DENATURED R-PHYCOERYTHRIN (R-PE) AND C-PHYCOERYTHRIN (C-PE) IN 2 M TRIS IN 5 M GUANIDINE-HCl, pH 8·9.

The multiplicity of absorbance maxima observed in some biliproteins probably results from the complexities of bilin-protein interactions. Claims for different chromophores based only on absorbance spectra maxima must be entertained with considerable reservation.[4]

EXPERIMENTAL

Reference phycoerythrobilin from C-phycoerythrin (ex. *Phormidium persicinum*) and phycocyanobilin from C-phycocyanin (ex. *P. luridum*) were prepared by previously described procedures.[1,2,4] *Rhodomonas lens*, *Chroomonas* sp. and *Porphyridium cruentum* were grown in a mass culture apparatus,[14] in synthetic marine media at 18°. *Grinellia americana* and *Ptilota serrata* were collected at Woods Hole, Massachusetts, and Halifax, Nova Scotia, respectively.

Cryptomonad phycoerythrin (ex. *Rhodomonas*), and phycocyanin (ex. *Chroomonas*), B-phycoerythrin (ex. *Porphyridium*) and R-phycoerythrin (*Ptilota* and *Grinellia*) were released from the algae by freezing and thawing in 0·1 M phosphate buffer, pH 7·0. The very small amounts of phycocyanins (C-phycocyanin and allophycocyanin) present with the phycoerythrins (except *Rhodomonas*) do not interfere with preparation of the

[14] H. LYMAN and H. W. SIEGELMAN, *J. Protozool.* 14, 297 (1967).

chromophores. The biliproteins were used without purification. The procedures for denaturation of biliproteins and liberation of the bilins have been previously described.[1,2,4] B-Phycoerythrin differs from all other biliproteins in not precipitating upon denaturation with 1–5 per cent trichloracetic acid. Precipitation was achieved by denaturing with 3 volumes of acetone. The cleaved phycoerythrobilins were methylated with diazomethane; the phycocyanobilin with 7 per cent BF_3-methanol. Preparative and analytical chromatographic systems, purification and crystallization procedures have been previously described.[1,2,4]

Visible-u.v. absorption spectra were recorded in 5 per cent HCl-methanol (w/v) and ethanol saturated with zinc acetate with a Cary 14 spectrophotometer. I.r. absorption spectra were recorded in KBr discs with a Perkin–Elmer Infracord.

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