PROzyme INC.

PHYCOBILIPROTEINS

SPECIFICATIONS

See individual Technical Data Sheets for each product.
Shipped with cold packs for next day delivery. Store at 4°C in the dark. DO NOT FREEZE.
Stability: Protein is supplied as a 60% Ammonium Sulfate suspension in 50 *mM* phosphate buffer. Stable at least 12 months when stored properly.

Phycobiliproteins are water soluble fluorescent proteins derived from cyanobacteria and eukaryotic algae. In these organisms, they are used as accessory or antenna pigments for photosynthetic light collection. They absorb energy in portions of the visible spectrum that are poorly utilized by chlorophyll and, through fluorescence energy transfer, convey the energy to chlorophyll at the photosynthetic reaction center. Other algal accessory pigments, which serve similar functions, are not water-soluble in their own right, but may be solubilized through their association with proteins.

Nomenclature: Phycobiliproteins are classified on the basis of their color into two large groups, the phycoerythrins (red) and the phycocyanins (blue). Absorption maxima for phycoerythrins lie between 490 and 570 nm while absorption maxima for phycocyanins are found between 610 and 665 nm. These large groups have been subdivided to reflect variation among the proteins in the exact location of the absorbance maximum and the specific shape of the absorbance spectrum.

Originally, these subdivisions, identified by letter prefixes to the phycobiliprotein name (e.g. C-phycocyanin, abbreviated C-PC) indicated the taxa of the organisms from which the pigments were isolated. For example, R-phycoerythrin (R-PE) was first isolated from the Rhodophyta. Further research has shown, however, that specific phycobiliprotein types are not always restricted to specific taxa. In fact, there is a full continuum of spectral types of phycoerythrins, determined by relative abundance of bilins (see below). They can be produced by different organisms or, on occasion, by the same organism under different growth conditions or different stages of its life cycle. Thus, letter prefixes applied to phycobiliproteins are currently only a general indication of the shape of the absorbance curve, and similarly named pigments isolated from different sources should not be assumed to be identical. In some pigments isolated more recently, the name is followed by a number which indicates the approximate absorption maximum (McColl and Guard-Friar, 1987).

Structure: The phycobiliproteins are composed of a number of subunits, each having a protein backbone to which linear tetrapyrrole chromophores are covalently bound. All phycobiliproteins contain either phycocyanobilin or phycoerythrobilin chromophores, and may also contain one of three minor bilins; phycourobilin, cryptoviolin, or the 697-nm bilin. Each bilin has

unique spectral characteristics, which may be further modified by interactions of the subunits and of the chromophore with the apoprotein.

The phycobiliproteins in many algae are arranged in subcellular structures called phycobilisomes. These structures allow the pigments to be arranged geometrically in a manner which helps to optimize the capture of light and transfer of energy. All of the phycobiliproteins absorb incident light directly, but in addition they participate in an energy transfer chain within the phycobilisome: phycoerythrin -> phycocyanin -> allophycocyanin -> chlorophyll a.

Physical properties: After isolation, phycobiliproteins have good long-term stability when stored refrigerated (2–5°C) as ammonium sulfate precipitates. Purified biliproteins may disassociate into subunits under acidic or basic conditions, but are relatively stable at room temperature at neutral pH and at concentrations greater than 0.1 mg/ml. Disassociated subunits typically have less intense coloration and fluorescence and are somewhat different in color than the native pigment.

Technical Data: The characteristics of eight water-soluble algal pigments are summarized below.

Properties of Phycobiliproteins

Pigment	Absorbance maximum[1] (nm)	Fluorescence emission[2] (nm)	Molecular weight[3] (kda)	Absorbtivity[4] (L/g-cm)	Molar absorptivity[4] $(M\text{-}cm)^{-1}(10^{-6})$	Fluorescence: Absorbance[5] (relative to R-PE)
R-Phycoerythrin	565 (495)	575	240	8.2	1.97	1.00
B-Phycoerythrin	545	575	240	10.0	2.40	1.40
Y-Phycoerythrin	~495 (545)	~563				0.50
C-Phycocyanin	615	647	220	7.0	1.54	0.15
R-Phycocyanin	617 (555)	637	100	7.0	0.70	0.14
Allophycocyanin	652	660	100	7.3	0.73	0.30
Phycoerythrin 566	566	617	55	8.0	0.44	0.25
Phycoerythrocyanin	575	625	100	8.5	0.85	0.50

[1] Values in parentheses indicate secondary absorbance maxima.

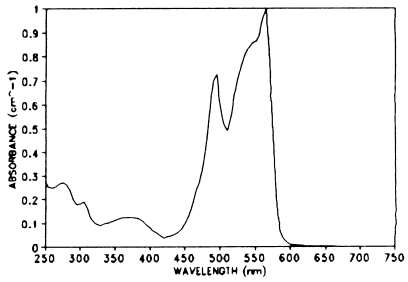
[2] Phycobiliproteins are aggregates of subunits, and various aggregates may occur in aqueous solution. Values given are for most common reported aggregates; aggregates of both larger and smaller size may occur.

[3] Value for Phycoerythrin 566 is an estimate.

[4] An approximate relative indicator of the quantum efficiency of the pigment (measured at absorbance and emission maxima).

R-Phycoerythrin

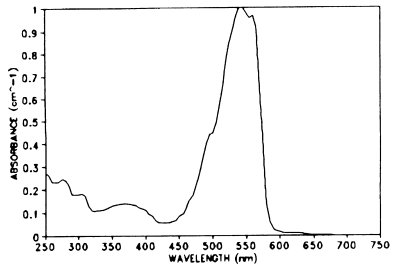
R-Phycoerythrin (R-PE) was originally isolated from red algae and has not been found in other taxa. It has its primary absorbance peak at 565 nm with secondary peaks at 496 and 545 nm. The relative prominence of the secondary peaks varies significantly among R-PEs from different species. R-PE has three types of subunits: α (~20,000 daltons), β (~20,000 daltons) and γ (~30,000 daltons). The molecular weight of intact R-PE has been found to be about 240,000 daltons, and a subunit structure of $(\alpha\beta)_6\gamma$ has been determined. The α subunit of R-PE contains only the phycoerythrobilin (PEB) chromophore, while the β and γ subunits contain both PEB and phycourobilin (PUB). Variability in the absorbance spectra of R-PEs from various species reflects differences in the PEB:PUB ratio of the subunits. R-PE and closely related B-PE are the most intensely fluorescent of the phycobiliproteins, with quantum efficiencies probably in excess of 90%, and its orange fluorescence is readily visible by eye in any moderately concentrated solution.



R-phycoerythrin

B-Phycoerythrin

B-Phycoerythrin (B-PE) has the same three absorbance peaks as R-PE, but its absorbance maximum occurs at 545 nm instead of 565 nm. The subunit structure of B-PE is also similar to that of R-PE, but the chromophore content of the subunits differs, causing the difference in the relative intensities of the absorbance peaks: the α and β subunits contain only PEB while the γ subunit contains PEB and PUB. B-PE is found both in cyanobacteria and red algae. By eye, the intense pink color and orange fluorescence of B-PE are virtually indistinguishable from those of R-PE.

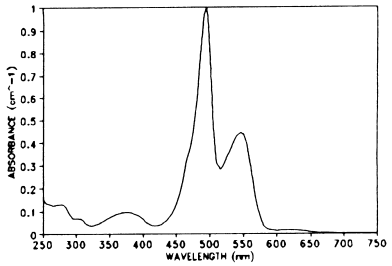


B-Phycoerythrin

Y-Phycoerythrin

PROZYME has recently added a new cyano-bacterial phycobiliprotein, to which we have assigned the designation Y-phycoerythrin (Y-PE) in recognition of the shift of its fluorescence emission toward the yellow, relative to R- and B-phycoerythrins. Its absorbance and excitation maxima are located at ~495 nm, making it particularly suitable for excitation with a 488 nm laser. Its shorter emission wavelength (~563 nm), relative to other phycoerythrins (575 nm), makes it a good candidate for multicolor fluorescence applications, where separation from higher wavelength emissions is desired. In addition, preliminary results suggest that

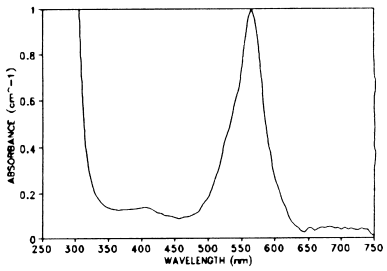
Y-PE may be a more efficient donor in fluorescence resonance energy transfer (FRET) applications. The protein contains alpha, beta and gamma subunits, but its molecular weight remains to be determined. The shift in its spectral characteristics, relative to other phycoerythrins, reflects a high content of the phycourobilin chromophore.



Y-Phycoerythrin

Phycoerythrin 566

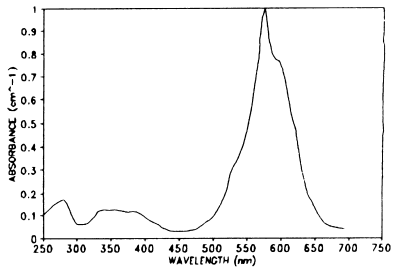
Phycoerythrin 566 (PE 566) is obtained from a unique group of organisms called cryptomonads. Unlike the other phycobiliproteins, these pigments are not organized into phycobilisomes within the organism. They appear to occur as $(\alpha\beta)_2$ dimers and show a single absorbance maximum at 566 nm. The chromophores of PE 566 are PEB and cryptoviolin (CV), but their distribution among the subunits is uncertain. Due to the presence of CV, the fluorescence emission maximum of PE 566 is shifted much further into the red than that of other phycoerythrins, to approximately 615 nm. The visual appearance of PE 566 is more lavender than that of the other phycoerythrins discussed here.



Phycoerythrin 566
(partially purified)

Phycoerythrocyanin

Phycoerythrocyanin (PEC) has been isolated from certain cyanobacteria, and is the only cyanobacterial pigment containing CV chromophores. In combination with phycocyanobilin (PCB) chromophores, this produces an absorbance maximum at 575 nm and emission maximum at 625 nm. A subunit structure of $(\alpha\beta)_3$ has been proposed for this pigment. It is lavender to the eye with red fluorescence.

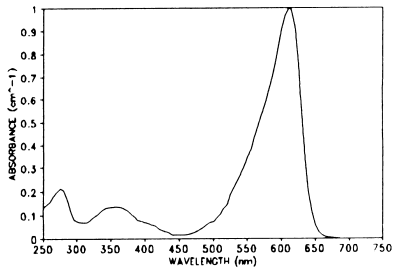


Phycoerythrocyanin
(partially purified)

C-Phycocyanin

C-Phycocyanin (C-PC) occurs as the major phycobiliprotein in many cyanobacteria and as a secondary phycobiliprotein in some red algae. The pigment has a single visible absorbance maximum between 615 and 620 nm and a fluorescence emission maximum at

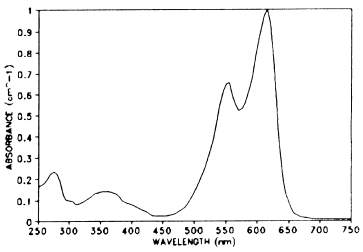
around 640 nm. Its molecular weight is between 70,000 and 110,000 daltons. The pigment is composed of two subunits, α and β, which occur in equal numbers, but the exact number of αβ pairs which make up the molecule may vary among the species. Both α and β subunits contain only the PCB chromophore. In addition to absorbing light directly, this intensely blue pigment accepts quanta from phycoerythrin by fluorescent energy transfer in organisms in which PE is present. The red fluorescence of C-PC is transferred to allophycocyanin (see below).



C-Phycocyanin

R-Phycocyanin

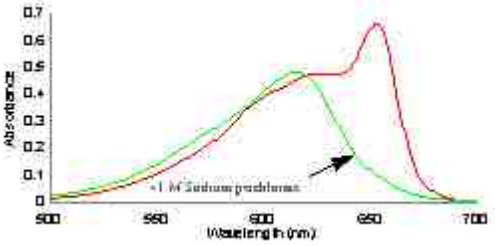
Like C-PC, R-Phycocyanin, (R-PC) has its absorbance maximum at 615 nm, but it also has a secondary absorbance maximum at 555 nm. This secondary maximum is due to the presence of a PEB chromophore on the β subunit (in addition to the PCB chromophores). The molecular structure of R-PC is thought to be $(\alpha\beta)_3$ and the molecular weight approximately 110,000 daltons. The PEB makes R-PC appear slightly more purple to the eye than the purer blue of C-PC, but it produces a similar red fluorescence.



R-Phycocyanin

Allophycocyanin

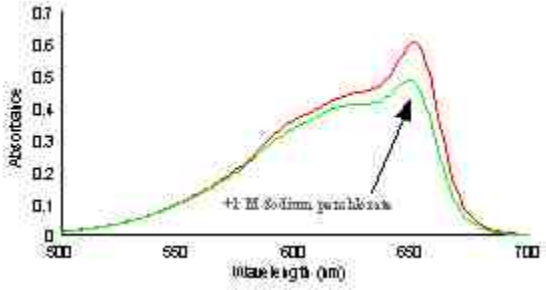
Several forms of allophycocyanin (APC) have been identified, depending on both the organism studied and the exact function of the individual molecule in the final transfer of energy from the phycobilisome to the chlorophyll reaction center. The most common form has an absorbance maximum at 650 nm. Like C-PC, APC carries only the PCB chromophore; its significantly different spectral properties result solely from conformation effects on the chromophores. It possesses α and β subunitss with an apparent structure of $(\alpha\beta)_3$, and is bright blue to the eye. PROZYME can supply APC from two distinct taxa, cyanobacteria and red algae. The A_{650}/A_{280} ratio, a measure of purity, is intrinsically lower in APC from red algae because of its lower number of chromophores per unit protein (probably because of a colorless protein in the aggregate).



Crosslinked APC

Allophycocyanin is the least stable of the major phycobiliproteins, susceptible to dissociation at low concentrations including concentrations at which some assays are performed. For this reason, many researchers prefer to use APC that is chemically cross-linked between the α and β subunits, which significantly retards dissociation of the complex.

Sodium perchlorate (1*M*) has been shown to be a particularly effective agent for disruption of the APC quaternary structure. This disruption causes changes in both the absorbance and emission spectra, including loss of the characteristic 650 nm absorbance maximum (see figure on previous page).



Disruption is virtually eliminated, however, in PROZYME's cross-linked allophycocyanin (see figure below). The accompanying table summarizes the values measured for the primary and secondary peaks with and without the chaotropic treatment and reports the ratio. These data clearly demonstrate the utility of crosslinking for maintaining the structural integrity of APC.

Source	sp	A_{615}	A_{650}	ratio
APC	–	0.45	0.65	1.43
	+	0.48	0.11	0.24
PROZYME xl APC	–	0.42	0.61	1.44
	+	0.39	0.48	1.22

Comparison of absorbance differences before and after treatment of APC and cross-linked APC with a chaotropic agent.

REFERENCES

Glazer, A. N. Phycobilisomes: structures and dynamics. **Ann. Rev. Microbiol. 36**:173–198 (1982).

Kronick, M. N. The use of phycobiliproteins as fluorescent labels in immunoassay. **J. Imm. Meth. 92**:1–13 (1986).

MacColl, R. and D. Guard-Friar. Phycobiliproteins. CRC Press, Inc., Boca Raton, Florida. (1987).

PROZYME INC.

1933 Davis Street, Suite 207
San Leandro, CA 94577-1258

TOLL FREE (800) 457-9444
PHONE (510) 638-6900
FAX (510) 638-6919

E-MAIL info@prozyme.com
WEB www.prozyme.com