

**BIOTECHNOLOGICAL POTENTIALS OF PHYCOBILIPROTEINS****RICHA, VINOD K. KANNAUJIYA, MINU KESHERI, GARVITA SINGH AND RAJESHWAR P. SINHA*****Laboratory of Photobiology and Molecular Microbiology, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005, India****RAJESHWAR P. SINHA****Laboratory of Photobiology and Molecular Microbiology, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005, India**

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ABSTRACT

Phycobiliproteins (PBPs) are a group of antennae-protein pigments involved in light-harvesting in cyanobacteria, rhodophytes, cryptomonads and cyanelles. The phycobiliproteins are organized in supramolecular complexes, called phycobilisomes (PBSs), which are assembled in regular arrays on the outer surface of the thylakoid membrane. They have antimicrobial, antioxidant, anti-inflammatory, neuroprotective and hepatoprotective properties. In addition, they are being extensively used as natural colorants in food and cosmetics, fluorescent neoglycoproteins, probes for single particle fluorescence imaging fluorescent applications in clinical and immunological analyses. However, a comprehensive knowledge and technological base for augmenting their commercial utilities is lacking. This review deals with the structure and biotechnological potentials of phycobiliproteins.



KEYWORDS

Allophycocyanin (APC), Phycocyanin (PC), Phycoerythrin (PE), Phycoerythrocyanin (PEC), Fluorescence activation cell sorter (FACS), Fluorescence resonance energy transfer (FRET), Single particle fluorescence imaging (SPFI).

INTRODUCTION

Phycobiliproteins (PBPs) are a family of accessory light-harvesting macromolecules organized in supramolecular complexes, called phycobilisomes (PBSs) that function as components of the photosynthetic apparatus in cyanobacteria and some eukaryotic algae¹. Captured light energy by PBSs complexes are transferred to the chlorophylls of the inner chlorophyll antenna, CP43 and CP47 (containing chlorophyll *a* and carotenoids) and finally to the reaction centre II. PBPs are nitrogen storage compounds and may constitute up to 60 % of the soluble proteins of the cell. PBSs may undergo changes in the ratio of phycocyanin to phycoerythrin in rods to improve light-harvesting in changing habitats². PBSs complexes are highly ordered; having supramolecular assemblies that carry covalently linked bilins (open-chain tetrapyrrole chromophores) and linker polypeptides.

Modern research and development in the synthesis and function of PBSs have expanded the potential applications of PBPs in biotechnology, diagnostic, food and medicine. They are extensively commercialized for fluorescent application in clinical and immunological analysis. This review mainly deals with the structural properties and biotechnological aspects of phycobiliproteins.

Structure of PBSs and PBPs

PBSs are multimolecular configuration made up of several polypeptide classes^{3,4}. PBPs, mainly composed of α & β polypeptides (some phycoerythrin have γ subunits) are bright colored systematic assembly of disc shaped proteins bearing covalently attached open chain tetrapyrrole known as phycobilins and are the

main components of PBSs⁵. α and β subunits associate into heterodimers and subsequently aggregate into trimers and hexamers. On the basis of color and spectral properties, PBSs are categorized into four groups; Phycocyanin (PC; λ_{\max} = 610-620 nm), Phycoerythrin (PE; λ_{\max} = 490-570 nm), Allophycocyanin (PE; λ_{\max} = 650-660 nm) and Phycoerythrocyanin (PEC; λ_{\max} = 560-600 nm)⁶. A number of colorless linker polypeptides maintain the overall structure of the phycobilisome complex as well as direct its assembly. Linker polypeptides not only serve as structural elements involved in the biosynthesis and stabilization of PBS⁷, but also facilitate efficient flow of excitation energy to the photosynthetic reactions centres. The membrane-phycobilisome association is mediated by a large chromoprotein present within the phycobilisome core, which also has linker polypeptide features; it is referred to as the anchor protein or core-membrane linker polypeptide^{2,8} (Fig. 1).

PC contains two subunits α and β , that are the products of *cpcA* and *cpcB* genes respectively. Some genes coding for PC and linker polypeptides in phycobilisome rods constitute the *cpc* operon⁶. Specific rod linkers (LR) assemble the PC hexamers into rods and tune their electronic properties in order to optimize directional energy transfer⁹. In the cyanobacterium *Synechocystis* sp. PCC 6803, operon contains five genes: *cpcB* and *cpcA* encode the β -PC and α -PC subunits, respectively, while *cpcC2*, *cpcC1* and *cpcD* encode the rod linkers L_R^{30} , L_R^{33} and L_R^{10} respectively and the two independent genes (*cpcG1* and *cpcG2*) encode the rod-core linker (LRC) that attaches the proximal PC hexamer to the core¹⁰.

Hemi-discoidal PBS present in the thylakoid membrane

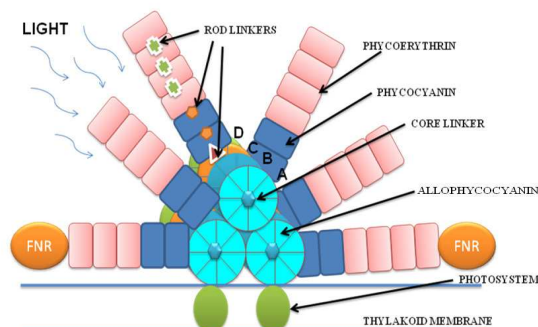


Figure 1

A hemi-discoidal PBS attached to the thylakoid membrane showing phycobiliproteins and associated linkers. FNR: Ferredoxin:NADP⁺ oxidoreductase.

Phycobiliproteins: biotechnological potentials

Phycobiliproteins have various commercial applications as depicted in (Fig. 2). Following are the brief documentation of applications of PBPs in various fields.

Commercial applications of phycobiliproteins

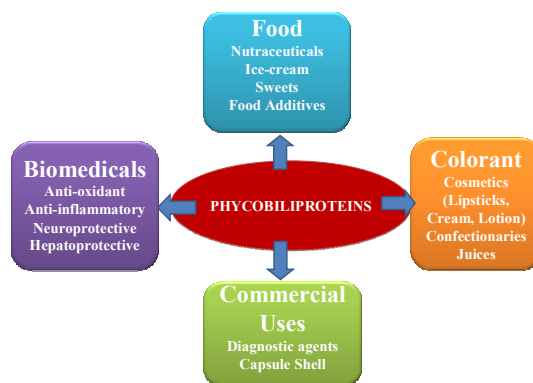


Figure 2

Phycobiliproteins and its potential commercial applications.

Fluorescence based applications of PBPs

The use of fluorescent probes as labels in immunoassay has increased dramatically in the current years (Fig. 3). Fluorescence immunoassay would be of great value if probes have the following properties (a) excitation and emission at the red end of the spectrum, where interferences from biological matrices tend to

be less (b) a large Stokes shift, so that interference from Rayleigh and Raman scatter and other fluorescing mechanism is less significant or missing (c) immunity from quenching by naturally occurring biological substances (d) high solubility in an aqueous environment so that nonspecific binding effects are minimal and (e) fluorescence quantum yield

independent of pH. Furthermore, they can easily be conjugated to specific molecules, thereby, being useful in the context of biological assay procedures.

The spectroscopic and structural properties of PBPs reveal several exceptional qualitative and quantitative characters such as (a) very strong and broad absorption in visible light spectrum and enormous extinction coefficient (b) high fluorescence quantum efficiency in a broad pH range values in neutral solution (c) large Stokes shift (d) very little fluorescence quenching between multiple chromophores that a PBPs carries and even between PBPs themselves (e) large surface functional groups that are readily coupled by using hetero-bifunctional reagents to variety of small organic dyes or proteins. Because of all these characteristics, PBPs are regarded as better suited fluorescent probes than small conventional synthetic labels and as ideal candidates for practical use. With the development of the commercial products of PBPs and their conjugates, the utilization of the

proteins as fluorescent probes has shown ever-extensive prospects in various practical applications of biology fields^{11,12}.

The PBPs used as fluorescent probes are basically required to have high purity level. They are >100 times more sensitive than conventional organic fluorophores. Even in practical applications such as flow cytometry and immunoassays, the sensitivity of PBPs-conjugated antibodies is generally much superior to that of the corresponding organic molecule-based conjugate. PBPs have numerous sites for forming stable conjugation to many biological and synthetic materials. Allophycocyanin is the least stable among the major PBPs, susceptible to dissociation at low concentrations.

Synthesis of conjugates of PBPs with molecules having biological specificity like immunoglobulins, protein A, biotin and avidin has been reported. It has been shown that PBPs conjugates are excellent reagent for two color fluorescence analysis of signal cell using fluorescence activation cell sorter (FACS)¹³.

Use of phycobiliproteins in fluorescence-based techniques

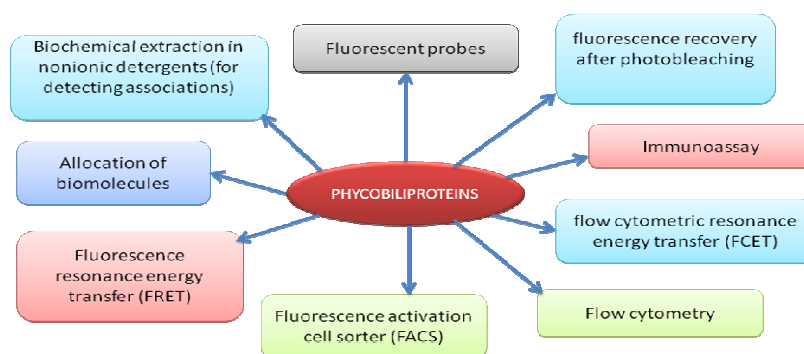


Figure 3

Utility of Phycobiliproteins in fluorescence-based applications

Following are the advantages of molecular fluorescence for bio sensing:

- The technique is exceptionally sensitive and there are increasing examples of even single-molecule detection using fluorescence methods¹⁴.

- Fluorescence measurements cause slight or no harm to the host system, thus providing the potential for completely non-invasive

sensing^{15,16}.



- Measurements can be made of not only fluorescence intensity but also fluorescence decay times¹⁵.
- Special fluorescence techniques can give information about the organization and micro-environment of molecules, and how these alter in response to analyte variations in health and disease^{17,18}.
- The structure and allocation of biomolecules can also be probed by the phenomenon of fluorescence resonance energy transfer (FRET)^{19,20}.

PBPs in immuno assay

Immunoassay techniques take advantage of the novel properties of PBPs. These stable, hydrophilic proteins can easily be linked to antibodies by conventional protein cross linking reagents. The PBPs are used as more powerful label for “sandwich” solid phase immunometric assays²¹. The increased absorptivity and low nonspecific binding allow a simple adaptation of a commercial system for determination of human immunoglobulin G (IgG) at picomolar sensitivity. In this application the PBPs fluorophore has certain spectral advantages related to its long-wavelength fluorescence and the large separation of its excitation and emission wavelengths²². Fluorescein and rhodamine were used as fluorescer and quencher, respectively, to transfer excitation energy from a fluorescein labelled antigen to a quencher-labelled antibody²³. The quenching is inhibited by competition from unlabeled antigen²².

PBPs as colorants

Now a days there is an increasing demand for natural colors which are of use in food, pharmaceuticals, cosmetics, textiles and as printing dyes. Due to toxic consequence of numerous synthetic dyes, there is an increasing liking to use natural colors for a range of uses. PBPs are used as a natural protein dye in the food production and in the cosmetic production. Phycocyanin is used as a natural tincture in food such as chewing gum, dairy products and jellies. In spite of its lower stability to heat and

light, phycocyanin is considered to be more adaptable than indigo and gardenia, presenting a bright blue color in jelly gum and coated soft candies²⁴. They are also used in coloring of many other food products such as fermented milk products, ice creams, soft drinks, desserts, sweet cake decoration, milk shakes and cosmetics. The shade of blue color produced from the red microalga *Phorphyridium aeruginosum* does not change with pH. The color was constant under light, but susceptible to heat, within a pH range of 4 to 5, the blue color produced is stable at 60°C for 40 min. This property was important for food uses, since many food items are acidic, particularly drinks and confections. The blue color was added to beverages without heat application which did not lose their color for at least 1 month at room temperature. The color was very stable in dry preparations. Sugar flowers for cake decoration maintained their colors for years of storage. Foods primed with the phycobiliproteins include gelatin and ice cream. In addition to its coloring properties, phycoerythrin possesses a yellow fluorescence. A range of foods that fluoresce under natural light and UV radiation were prepared and tested. Fluorescent color has also been added to alcoholic beverages containing up to 30% alcohol but the shelf life for such products is short. C-PC from *A. platensis* is manifested as a food and cosmetics colorant in Japan²⁵. Limited consumption of blue food has probably limited the industries interest in C-PC for food coloring. A small number of studies have addressed the functionality of C-PC in food with regards to color stability^{26,27}.

PBPs as biomedical agents

The biomedical properties credited to phycocyanin include antioxidant, anti-inflammatory, neuroprotective and hepatoprotective (Fig. 4). When it was evaluated as an antioxidant *in vitro*, it was able to scavenge alkoxyl, hydroxyl and peroxy radicals, and inhibits microsomal lipid peroxidation induced by Fe⁺² ascorbic acid or the free radicals initiators 2, 2' Azobis (2-amidinopropane) dihydrochloride, (AAPH). They also reduced edema, histamine (Hi)

release, myeloperoxide (MPO) activity and the levels of prostaglandin (PGE2) and leukotriene (LTB4) in the irritated tissues. Phycocyanin also reduces the levels of tumour necrosis factor (TNF- α) in the blood serum of mice-treated with

endotoxin and it showed neuroprotective effects in the rat cerebellar granule cell cultures. *Aphanizomenon flos-aquae* (AFA) as a source of phycocyanin have been described as a strong antioxidant^{28,29}.

Biomedical applications of phycobiliproteins

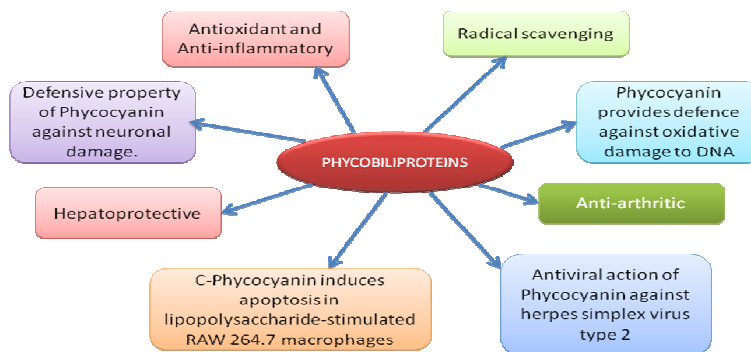


Figure 4
Biomedical properties of phycobiliproteins.

The participation of phycocyanin in the antioxidant defence of the AFA extract against the oxidative damage was demonstrated *in vitro*³⁰. In red blood cells, oxidative hemolysis and lipid peroxidation are induced by aqueous peroxy radical generator. In plasma samples, they inhibited the extent of lipid oxidation induced by pro-oxidant agent cupric chloride (CuCl₂). These findings about the phycocyanin consider the probable benefits in the prevention of many pathological disorders associated with oxidative stress and inflammation. Allophycocyanin was found to inhibit enterovirus 71-induced apoptosis. In addition, inhibitory effect of phycocyanin from *Spirulina platensis* on the growth of human leukemia K562 cells in a dose- and time-dependent manner was reported³¹. C-phycocyanin derived from *Spirulina platensis* powerfully influenced serum cholesterol concentrations and imparted a stronger hypocholesterolemic activity³². Phycocyanin also exerts hepatoprotective and anti-inflammatory effects in a human hepatitis animal model. It reduced alanine amino transferase (ALT), aspartate amino transferase (AST) and malondialdehyde (MDA) in the

serum³³. C-phycocyanin also suppressed the 3, 3', 5-triiodothyronine (T₃) induced increase in serum nitrite levels and in the activity of hepatic nitric oxide synthase (NOS)³⁴. Also, *in vitro* studies propose that *Spirulina* possesses antiviral activities³⁵⁻³⁷. *Spirulina* is also a dominant stimulant for the immune system, as shown in animal experiments, by increasing the phagocytic and the natural killer activities³⁸. In addition to this, hypocholesterolemic effects have been reported in some animal studies^{39,40}. *Spirulina maxima*, administered intraperitoneally, have shown to appreciably decrease carbon tetrachloride-induced hepatotoxicity⁴¹. Now a days, there has been a much interest in the use of antioxidant food supplements^{42,43}. Epidemiological proof suggests that intake of some vitamins, minerals, and other food constituents may help to protect the body against heart disease, cancer and the aging process, and that antioxidants may have a protective effect, either in preventing these diseases or decrease their severity⁴². Some activities of the antioxidants are mediated by inhibition of reactive oxygen



species (ROS), which are generated during the oxidative burst.

CONCLUSION

PBPs in the PBSs have unusual spectroscopic properties that have advantage over many organic fluorescent dyes. These properties make PBPs a promising fluorescent probe for medical labelling. Allophycocyanin is emerging as one of the brightest fluorescent probes. PBPs have high quantum yields that are

constant over a broad pH range. PBPs such as phycoerythrin and phycocyanin have been broadly used in staining of DNA and in diagnostic studies. Fluorescent labelling of biomolecules has been demonstrated as an indispensable tool in many biological studies. Anti-inflammatory effect of phycocyanin has recently been confirmed. There is need to advance thermal stability, aqueous, pH and light stability, alcohol resistant and shelf life of PBPs with a range of bioprocess engineering for the welfare of humans.

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