The Radiant World of Cyanobacterial Phycobiliproteins: Examining Their Structure, Functions, and Biomedical Potentials

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Abstract: Phycobiliproteins (PBPs) are accessory light-harvesting pigment complexes found in cyanobacteria, red algae, and certain types of cryptophytes. The unique spectral features (strong absorbance and fluorescence), proteinaceous nature, and some imperative properties such as the anti-oxidative, hepato-protective, anti-inflammatory, and anti-aging activity of PBPs allow their use in biomedical industries. However, basic research and technological innovations are required to explore their potential in biomedical applications. The techniques responsible for therapeutic effects need to be standardized for medical application purposes. This review focuses on the current status of PBPs, their structure, functions, methods of preparation, and applications. Additionally, the stability, bioavailability, and safety issues of PBPs, along with their use in therapeutics, are discussed.

Keywords: bioavailability; biomedical; cyanobacteria; phycobiliprotein

1. Introduction

The photosynthetic apparatus of cyanobacteria is composed of three primary light-harvesting systems: two photosystems common to other photosynthetic organisms and a phycobilisome (PBS). Phycobilisomes (PBSs) are large antenna complexes found in cyanobacteria that are crucial for photosynthesis. They capture light energy in the range of 450–650 nm, transfer it within its structure in a unidirectional way, and deliver it to the chlorophyll molecule of photosystems [1]. Although the PBSs of cyanobacteria are mainly composed of phycobiliproteins (PBPs) and linker proteins, their composition may vary from individual organism to different species. PBPs are in charge of absorbing and transmitting light energy, whereas linkers enable proper PBS assembly and control energy transmission [2]. Cyanobacterial PBPs are large, water-soluble supramolecular protein aggregates that are important accessory pigments during photosynthesis. Based on their spectrum features, PBPs can be generically classified into three classes: phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC). PBPs are commercially produced from *Spirulina platensis*, *Anabaena* sp., and *Galdieria sulphuraria* and have been extracted and purified from *Spirulina*, *Synechococcus*, *Oscillatoria*, etc. [3]. The structure, amino acid composition, abundance, and types of PBPs of cyanobacteria and algae vary depending on the species and the habitat in which they grow. The spectral properties of PBPs result largely from the environment of the chromophore conferred by the apoprotein rather than the structural properties of the chromophore itself. PBPs in cyanobacteria account for 50% of total cellular protein [4].

Each PBS has two distinct substructures: the rods and the core [5,6]. The core is made up of APC. Several rods made up of PE and PC extend from the core in an outward orientation, which further functions as the light-harvesting antennae. Certain cyanobacteria species, including *Spirulina platensis*, have rods that solely contain PC and protein linkers that are nonetheless connected to the PBS core [7]. APC and linker proteins combine to
create the core. This core transfers the energy captured by the rods to the chlorophyll present in the thylakoid membrane [8].

A growing interest in the study of microorganisms like cyanobacteria has resulted from the search for bioactive compounds in recent decades. This is because these organisms may have commercial applications in a variety of fields, including nutrition, animal and human health, wastewater treatment, energy production, and the chemical and pharmaceutical industries [9]. More specifically, cyanobacterial PBPs have widespread biotechnological applications due to their potent biological and pharmaceutical properties. PBPs, especially PC, have commercial and industrial applications due to their primary functions, including nutraceutical and therapeutic values (due to their pharmacology and biological activities, including anticarcinogenic, antioxidative, and anti-inflammatory activities, as well as a protective effect against various conditions), over-the-top fluorescent properties (high quantum yield, high Stokes shift, and an essential insensitivity to quenching), and natural colorants [10]. PBPs are useful fluorescent tags that find wide-ranging uses in immunoassay, reactive oxygen species (ROS) detection, flow cytometry, and fluorescence-activated cell sorting. These uses take advantage of PBP’s special spectroscopic and physical characteristics. They have a broad range of biomedical applications, such as in food coloring, biotechnology, medicines, cosmetics, and nutraceuticals [11]. The review aims to highlight the spectral and structural features of cyanobacterial PBPs along with their biotechnological and biomedical applications.

2. Spectroscopic and Structural Characteristics of Cyanobacterial Phycobiliproteins

Based on their absorption, PBPs have been divided into three major categories: APC ($\lambda_{\text{max}} = 650–660$ nm), PC ($\lambda_{\text{max}} = 610–625$), and PE ($\lambda_{\text{max}} = 490–570$) [12]. The most diverse chromophores among PBP are found in PE, which has a vibrant pink color. PE can be found in the PBS rods’ distal region. At 542 nm, C-PE exhibits a single absorption maximum. At a maximum emission value of 575 nm, the fluorescence emission of the various types of PE does not demonstrate significant variations [13]. To date, Phycoerythrocyanin (PEC) is exclusively reported in the photosynthetic membrane of *Mastigocladus laminosus*. It is a PBP with a purple-blue tint. One of the primary features of PEC is that during growth, its production is highly dependent on the kind and intensity of light. A significant increase in abundance was seen under low light conditions with green light [14]. The proximal portion of PBS rods contains the bright blue protein known as PC. There is just a single absorption maximum at 615 nm (Figure 1) [15]. APC, which is only present in the PBS core, is a bright turquoise tint with a maximum absorption of 650 nm [8]. A highly effective energy transfer in the PBS is made possible by these spectroscopic features of PBP absorption and emission.

2.1. Subunits and Heterodimers ($\alpha \beta$)

The basic component of PBPs is its subunits, specifically $\alpha$ and $\beta$ subunits, which stabilize each other to form an $\alpha \beta$ heterodimer. The self-assembly of all PBPs appears when two subunits, $\alpha$ and $\beta$, dock together [16,17]. This $\alpha$ and $\beta$ heterodimer is known as the fundamental component of PBPs’ monomeric structure. It subsequently comes together to form trimeric discs ($\alpha \beta$)$_3$. Monomer subunits are primarily distinguished from native trimeric units by dissociated subunits, which are usually less intense in color. In various PBPs, native trimeric and hexameric units have different molecular weights. When it comes to capturing solar light energy, the hexamer assembly is more functional and stable. On the other hand, the trimeric form of the majority of PCs has demonstrated an intriguing rod structure assembly without a requirement for linker polypeptides [18]. Four trimers of APC biliproteins come together to form core cylinders when they are assembled. A full functional structure is then formed by the subsequent assembly of two to five core cylinders into the core substructure with the help of linker polypeptides, as in Figure 2.
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The basic component of PBPs is its subunits, specifically α and β subunits, which stabilize each other to form an αβ heterodimer. The self-assembly of all PBPs appears when two subunits, α and β, dock together [16,17]. This α and β heterodimer is known as the fundamental component of PBPs’ monomeric structure. It subsequently comes together to form trimeric discs (αβ)₃. Monomer subunits are primarily distinguished from native trimeric units by dissociated subunits, which are usually less intense in color. In various PBPs, native trimeric and hexameric units have different molecular weights.

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Ultimately, a complete PBS is formed by the association of both core cylinders and rods (Figure 3). When it comes to high-resolution views of proteins, X-ray crystallography has emerged as an extremely valuable instrument. Initially, the entire structure of PBS was explored at low resolution through electron microscopy. PBS crystal structures have also significantly improved our potential to analyze the tertiary, or hexameric, structure of proteins as well as their stability and functionalities in a range of environmental circumstances [19].
2.2. PBPs Chromophore

Chromophores are open-chain tetapyrroles covalently linked to cysteine via thioether bonds. PBPs can vary in the number of chromophores they contain. Based on their structural characteristics, the chromophores can be categorized into the following categories: phycocyanobilin (PCB), phycoerythrobilin (PEB), phycoerythrobilin (PVB), or phycourobilin (PUB) (Figure 4) [21]. Each biliprotein consists of the α and β subunits that are linked through covalent bonds to the apoprotein’s cysteines through a thioether bond to C-3 on ring A, and occasionally through an extra thioether bond to C-18 on ring D. The chromophore that is developed from heme is joined to the apoproteins of bilin by an ethylidene bond, which adds a thiol group [22]. A single cysteiny1 thioester linkage via the vinyl substituents on the pyrrole ring A of the tetapyrrole, or periodically two cysteine linkages through the vinyl substituents on both the A and D pyrrole rings, are typically responsible for binding chromophores to the polypeptide chain at the conserved position. The assembly of chromophores within the clearly identified geometries of proteins’ cores determines the properties of light-harvesting PBPs in photosynthetic cyanobacteria [23]. The electronic state of chromophores and related apoproteins plays a major role in the establishment of the stable chemical structure of proteins. Consequently, the chromophore serves as a report on the integrity of the protein structure; the protein possesses a stunning dark blue color when it is in its native form, but this color disappears when it is denatured [24]. PBPs have been studied using fluorescence techniques because, when detached from reaction centers, they show strong fluorescence. The fluorescence quantum yield of the tetapyrrole chromophore in C-PC is roughly 60%, with a maximum fluorescence of 650 nm when it is maintained in a linear alignment by the protein scaffolding [25].
The heterodimeric (αβ) protomer of C-phycocyanin (C-PC) contains three PCB chromophores at cysteines α-84, β-84, and β-155. This residue is also conserved in the β subunit of the C-PC chromophore, where it is classified as Asp39 in the β-155 chromophore and Asp87 in β-84. It is present in both C-APC and C-PE. The chromophore’s electronic state is established by an important amino acid called the Asp87 residue [26]. Position Cys84 on the α subunit contains a single PVB chromophore, while positions Cys82 and Cys153 on the β subunit contain two PVB chromophores. PEB comprises a set of two to three covalently linked tetrapyrrole chromophores integrated with the α (164 amino acid) and β (177 amino acid) subunits of C-PE, respectively [27].

2.3. Linker Polypeptides

The PBP structure involves the non-pigmented linker polypeptides [28]. The polypeptides have molecular masses varying between 8 and 120 kDa. Two possible functions of linker polypeptides in the PBS have been proposed: (1) maintain the PBS structures and establish structural links between the nearest PBPs, and (2) adjust both the properties of absorption and fluorescence to support or directly take part in the energy transfer from the rod to the core and ultimately to the thylakoid membrane of the photosynthetic cells,
which contain chlorophyll [29]. The two basic subunits (\( \alpha \) and \( \beta \)) of PBPs along with its heterodimer, i.e., \( \alpha\beta \) are represented in Figure 5.

![Figure 5. Protein model of PBPs. (A) \( \alpha \) subunit, (B) \( \beta \) subunit, and (C) \( \alpha\beta \) heterodimer.](image)

The standard acronym for linker polypeptides is \( L_X^Y \), where \( X \) represents the location and \( Y \) represents the molecular mass of the linker polypeptide \( L \) PBS complex. In addition, \( X \) can be assigned either to the letter \( R \) (rod) or \( C \) (core) for the main chain, while \( R-C \) (rod-core) and \( CM \) (core-membrane) are used to represent junctions. In class I and II of \( R-P-E \), the \( \gamma \)-subunits and the core-membrane linker (LCM) PCB-ApcE are two of the linker polypeptides that contain covalently attached chromophores, while the majority of them are colorless [30]. Based on their location and functional characteristics, the linker polypeptides are divided into four groups. PBS: Group I consists of \( L_\alpha \) polypeptides (27–35 kDa) involved in the peripheral rod assembly, together with a small number of 10 kDa rod linker polypeptides (\( LR^{10} \), \( LR^{33} \), and \( LR^{35} \), for example, which connect trimeric or hexameric PC/PE structural components into rod segments); Group II \( L_{RC} \) polypeptides, which are between 25 and 27 kDa, take part in establishing the peripheral rods to the core subunits; LC polypeptides (8 kDa) belonging to Group III are essential for joining core components and ensuring their functional properties; and the main terminal energy emitter for PSII is Group IV, LCM (70–120 kDa), with a greater molecular weight of polypeptides that connects PBS to the photosynthetic membrane [31]. Six conserved domains (N-terminus) in rod linker polypeptides have been recognized by structural motif analysis as being crucial for the packing and assembly of rod discs into hexamers (Figure 3). There are connections between PBPs and linker polypeptides because the surfaces of linker polypeptides possess a positive charge, while the majority of globular proteins are probably hydrophobic [29].

3. Biomedical Potential of PBPs

In addition to their role in nature, PBPs have several biomedical applications. In Japan, China, India, and other European and Asian nations, PBPs are regarded as one of the most promising food products that people are using effectively as additives and marketing as food and cosmetic colorants [32]. In addition to PBPs' nutritional value,
they also possess antiviral, antioxidant, hepatoprotective, anti-inflammatory, and immune-stimulating characteristics (Figure 6). Here are some biomedical applications of PBPs.

3.1. Antioxidant Properties

PC may prohibit the peroxidation of lipids by constraining the oxidative activity of several kinds of radicals, including peroxyl, hydroxyl, and superoxide. It is possible that the metabolic response to oxidative stress may have an impact on cellular elements such as proteins, nucleic acids, and the cellular membrane itself, which can lead to a variety of illnesses, including cardiovascular disease, diabetes, cancer, inflammation, degenerative diseases, ischemia, and anemia (Figure 6) [33]. Several phytochemicals, such as α-tocopherol, caffeic acid, zeaxanthin, etc., are currently being utilized in the prevention and treatment of these kinds of diseases. Additionally, since PCB is as effective as other phytochemicals, Hirata et al. [34] suggest that it has a high potential for antioxidant properties. The organisms produce anti-oxidant compounds as a defense mechanism to protect themselves against oxidative effects. ROS accumulation is the main triggering factor of oxidative stress. Both enzyme-based and non-enzyme antioxidative processes deal with the neutralization of ROS. In order for compounds like PC to function as non-enzymatic antioxidants, they typically scavenge free radicals from ROS, neutralize reactive molecules, and reduce the level of oxidation. Particularly, the effects of oxidative stress can destroy DNA and result in apoptosis or mutagenesis. Both PC and PCB have been demonstrated to be effective at scavenging peroxynitrite and prohibiting damage to DNA [35]. According to Strasky et al. [36], phycocyanobilins have structural similarities with the human bile pigments biliverdin and bilirubin, the former of which serves as the most effective endogenous antioxidant substrate. However, due to the insolubility of water, the scarcity of natural sources, and the challenges of synthesizing it, bilirubin is not appropriate for oral administration. It is interesting that PCB has recently been proposed as a potential mimic molecule with atheroprotective properties [36].

Additionally, according to the Oxygen Radical Absorbance Capacity (ORAC) method developed by Benedetti et al. [37], PCB (as well as PC) has a strong antioxidant capability, which suggests an effective antioxidant profile for particular biological samples. A number of research studies have additionally revealed that PBPs possess the ability to scavenge free radicals [38]. Additionally, PC has been reported by Datla [39] as having strong antioxidant, immune boosting, and anti-inflammatory properties. In rodent models of autoimmune encephalomyelitis, Cervantes-Llanos et al. [40] demonstrated an application of PC as a neuroprotector, whereby PC decreased the level of oxidative stress and the response of the immune system. According to research by Gdara et al. [41], PC reduces liver damage by inhibiting the activity of oxidative stress-activated enzymes like alkaline phosphatase and liver transaminases. The liver enzymes P450, aminopyrine-N-demethylase, and glucose-6-phosphatase can all be protected by PC, which can significantly decrease liver toxicity. To preserve the liver enzymes, PC is therefore essential as a hepatoprotective. Moreover, by inducing thioacetamide, PC’s antioxidant property reduces hepatic brain injury [42]. Additionally, PC is already accepted for use as a food colorant and is utilized in cosmetic products as well.

3.2. Anticancer Properties

In addition to being one of the leading causes of death around the world, cancer is one of the most problematic diseases. To lessen the issue caused by cancer, several initiatives have been developed using various strategies. Over the past few decades, research has focused on natural products in the form of extracts and pure compounds from plants, algae, fungi, and cyanobacteria, with extremely positive outcomes [43]. Phycocyanin, in particular, is one of the cyanobacterial pigments that has been shown in the last few decades to have anticancer properties in a variety of cancer cell types such as liver, leukemia, melanoma, breast, etc., using both in vitro and in vivo assays [44]. Proliferation, deregulated apoptosis, and hyperplasia are the three fundamental features of cancer cells that can be used to
describe the mechanisms of action of an anticancer compound. Thus, the anticancer medication may prevent the growth of new cells and/or trigger the death of tumor cells. By modifying the growth of different tumor cell lines at different levels of their molecular mechanisms, PC exhibits the highest level of activity. According to recent theories by McCarty and associates, phycocyanobilin inhibits ROS production in the mitochondria, which prevents pancreatic cancer, and PBP can prevent cancer cachexia by reducing TLR4 signaling in skeletal muscles [45]. Furthermore, by lowering RIPK1/NF-κB and TIRAP/NF-κB activity, C-PC inhibits the in vitro proliferation and migration of non-small-cell lung cancer cells [46]. Normal tissue cells exhibited virtually no or very little proliferative effects from PC, and normal cell viability is unaffected by high concentrations of PC. Ying et al. in 2021 reported that PEs are also crucial as a potential treatment for human ovarian cancer by inhibiting growth and inducing apoptosis in SKOV-3 cells. The ROS/JNK/Bcl-2 signaling pathway, upregulation of JNK, GADD45A, RAD23, and downregulation of XBP1 and OS9 are critical in PE-induced apoptosis in this cell [47].

3.3. Anti-Inflammatory Effects

Whenever there is an internal or external injury or infection, the immune system responds dynamically with inflammation [48]. PBPs, particularly PC, have been demonstrated to exhibit a number of anti-inflammatory properties. In general, an inflammatory response is an essential aspect of everyday life for people. This process may happen with minor symptoms like discomfort in the muscles or more serious conditions like acute lung injury, which is identified by injury to the epithelial and endothelial cells in the pulmonary system and can be lethal for patients in intensive care units (ICUs) [49]. Inflammatory disorders typically have macrophage induction as their primary source of pro-inflammatory signals. The primary mechanism by which PBPs exert their anti-inflammatory effects is through the stimulation and expression of enzymes, the modulation of macrophage function, and the suppression of pro-inflammatory signals. Depending on the type of infection, different target tissues and pro-inflammatory signals are produced. Additionally, the inhibition of two key signaling pathways, nuclear transcription factor-κB and mitogen-activated protein kinases (MAPKs), which play a significant role in the synthesis of numerous pro-inflammatory mediators, is known to be the cause of the anti-inflammatory activity of biologically active extracts and natural substances [33]. There are various ways that PBPs, and especially C-PC, can function. Since cyclooxygenase (COXs) (1 and 2) play a role in the production of significant biological mediators, known as prostanoids, which are directly connected to the inflammatory process, anaphylactic reactions, and vasoconstriction, they can provide relief from the symptoms of inflammation and pain by reducing the activity of COX-2 [50]. Additionally, C-PC was suggested as a radiosensitizing agent to lessen patient radiation therapy countermeasures because colon cancer radiation therapy hinders COX-2 expression (Figure 6) [51]. Furthermore, the PBPs inhibit the synthesis of hydrogen peroxide and hypochlorous acid, which oxidatively damage the host tissue, by inhibiting the performance of the myeloperoxidase enzyme (MPO) [2]. By inhibiting cofactors like NF-κB, PC can regulate apoptosis and inflammatory reactions. NF-κB is an important factor in controlling the immune system’s reaction to infection, but its abnormal activity has been connected to autoimmune, inflammatory, and cancerous conditions. PC is useful in treatments and as a prophylactic, since it inhibits a portion of the cofactor [52]. Furthermore, Kim and colleagues [53] demonstrated the application of PC to inhibit UV-induced epithelial apoptosis. The process is induced by a protective cascade and is under the control of protein kinase C (PKC).
PC has recently been used to determine anti-inflammatory properties, including the in vitro inhibition of albumin denaturation, anti-proteinase, hypotonicity-induced hemolysis, and anti-lipoxygenase activities \[55\]. However, in an immunosuppressive model induced by hydrocortisone (HC), R-PE was found to have immunomodulatory activity in both the innate and adaptive immune systems through TLR4/NF-κB-dependent immunocyte differentiation \[56\].

Additionally, Gonzalez et al. \[57\] suggested that PC plays an important role in treating several diseases and alleviating the symptoms of a variety of inflammatory processes.

3.4. Potential as Diagnostic Tools

PBPs are distinctive and beautifully colored proteinaceous photosynthetic pigments. In both medical and non-medical diagnostics, PBPs are frequently utilized as fluorescence-detecting systems. Primarily, its use was limited to the diagnosis of human infectious illnesses, metabolic abnormalities, malignancies, and immunological disorders. Later on, their use was expanded further to include the prognosis of a few human illnesses. PBPs are important in the innovation of human diagnostics, either as fluorescence-detecting elements or as both diagnostic systems and therapeutic molecules. They have been heavily employed in the identification of cell lineages, cell components, and cell subsets, as well as other biological and environmental probes.

Protein conjugates, molecular tags, antibody conjugates, fusion proteins, and DNA probes are only a few of the detecting probes that have been the subject of substantial patented research. Additionally, PBPs are incorporated into microbeads, magnetic beads, and biochips. Around the world, several businesses sell diagnostic PBPs in the form of preparations like streptavidin conjugates, activated PBPs, FITC-crosslinked forms, purified and activated forms, and multi-color detection systems with custom designs. Among all, PE is the most widely used PBP, followed by APC and PC.

PBPs continue to be extensively used for the detection and diagnosis of several metabolic diseases and syndromes in humans. For instance, the expression of integrin IIb-3 can be used to determine the functional capacity of platelets in thrombosis and hemostasis.
On resting platelets, its expression was not strong. The monoclonal antibody (mAb) against Integrin IIb-3 was conjugated to R-phycoerythrin (R-PE) in this diagnostic method [58]. PBPs are now applicable to environmental problems as well. The detection of diarrhetic shellfish poisoning toxin, Okadaic acid (OA), produced by toxigenic dinoflagellates, was performed using magnetic beads coated with streptavidin. It prevents hazards to public health [59]. The detection of Cu\(^{2+}\) ions based on the quenching effect between R-PE-AgNPs and Cu\(^{2+}\) ions, in conjunction with the fluorimetric approach, was made using the R-PE-AgNPs (silver nanoparticle) construct [60].

PE, as a fluorochrome, was used to detect the amyloid protein (A), which diagnoses Alzheimer’s disease [61]. Similar to this, hemophagocytic lymphohistiocytosis showed down-regulated CD5 expression on activated CD8+ T cells utilizing an immunofluorescence kit including the appropriate antibody-coupled PE and other colors [62]. PBPs might be used as a component of detection in a range of human malignancies after the diagnosis of human pathogenic and non-pathogenic disorders. The imaging of cancer development and therapy effectiveness using PC-attached nano horns was successful in vivo. It is noteworthy that PC was previously shown to mediate the formation of ROS photodynamically to destroy cancer cells [63].

In one such implementation, CD5-FITC/CD19-PE immunostaining was used to identify chronic lymphocytic leukemia [64], where FITC was a cross-linker. The recombinant fusion protein streptavidin-phycobiliproteins (SA-PBP’s) in Sandwich ELISA was used to identify liver cancer using the biomarkers -fetoprotein and carcinoembryonic antigen. According to Ge et al. [65], the protein streptavidin is derived from Streptomyces avidinii. Epithelial cell adhesion molecule (EpCAM), which is present in the cell membrane, was detected in live breast cancer cell lines using multicolor staining with 4,6-diamidino-2-phenylindole (DAPI) for the nucleus and an APC-labeled anti EpCAM antibody (Table 1) [66].

Streptavidin-phycobiliprotein conjugates a recombinant fusion protein, which was made in E. coli via combinational biosynthesis. SA-PCA-PEB (streptavidin-phycocyanin subunit phycoerythrobilin) and SA-PCA-PCB (streptavidin-phycocyanin subunit phyco-cyanbilin) were two recombinant fusion proteins. In E. coli, a dual plasmid system was used to biosynthesize another recombinant fusion protein called holo-ApcA, which contained streptavidin and the APC-subunit. An in vitro chromophore attachment reaction system including PCB and lyase gene cpcS providing the robust signal for detection was used to improve the rate of chromophorylation of the fusion protein with the prosthetic group PCB [67]. It has been conducted using a similar system of fusion protein with tandem repetitions of APC holo-subunits to increase fluorescence brightness [68].
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<td><a href="http://ipindia.gov.in/">http://ipindia.gov.in/</a> (15 November 2023)</td>
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**Abbreviations:** IN—InPASS database, India; US—USPTO database, United States of America; CN—CNIPA database, China; (E)—Espacenet database, European Patent Office; JP—J-Plat Pat database, Japan; KR—KIPRIS database, Korea. Patent numbers represent application numbers, except numbers with the prefix “CN” and suffix “(E)” that denote publication numbers. Except for Espacenet, numbers alone (without prefix alphabets) have to be entered in the suitable search field of the corresponding country’s database. Such prefix alphabets are meant for identifying the database. In Espacenet alone (tagged with “(E)” as a suffix of numbers), given alphabetic prefixes along with the publication numbers must be fed to access patent details.
3.5. Application in Photodynamic Therapy (PDT)

PDT is a method that causes cancer cells to die by producing free radicals by strong sun radiation and photosensitizers [70]. According to Bharathiraja et al. [71], PDT is a non-invasive, cutting-edge approach to treating infectious illnesses caused by bacteria and viruses, psoriasis, and other terrible, incurable conditions. Compared to localized surgery and traditional chemotherapy and overcoming drug resistance mechanisms such as antibiotics, this treatment offers several benefits. Numerous photosensitizers, including protoporphyrin IX produced from chlorophyll and chlorine 6, have been identified; however, because of their hydrophobic nature, they clump readily in physiological solutions, which lessens the effectiveness of the photodynamic therapy mechanism [72].

Due to its high water solubility, nontoxicity, and immune-modulating qualities, PC offers several benefits over photosensitizers generated from chlorophyll [73–76]. PC might be utilized in PDT for the elimination of cancer without causing any harm to normal cells since it is easily metabolized in normal cells as opposed to diseased cells [71]. Low-level laser treatment (LLLT), a nonthermal irradiation, has recently been created to reduce pain and inflammation during the PDT process (Figure 7). According to Avci et al. [77], this cutting-edge technology has been utilized to treat a wide range of illnesses, including traumatic brain disorders, myocardial infarction, tumor removal, and other cancer-related conditions. Following PC-based PDT treatment, cancer cell lines such as HeLa tumor and MCF-7 breast cancer cells have demonstrated tumor cell death and immunological enhancement [78,79].

Figure 7. A schematic model for photodynamic therapy treatment, modified from [3].

4. Phycobiliproteins as Bioluminescent Markers

The disintegration or production of tetrapyrrole macrocycles produces the open chain tetrapyrroles, also known as bilins. These days, we know that bilins are involved in many different biological processes, including signaling, photomorphogenesis, redox chemistry, and light-harvesting (in PBSs). Depending on the degree of conjugation, bilins’ absorption spectra cover the visible, ultraviolet, and near-infrared (NIR) areas, resulting in a broad spectrum of colors ranging from red/orange to blue/green. Since bilins frequently have
very low fluorescence intensities, there are fewer accessible spectra. However, structural rigidification can significantly improve fluorescence, as shown by the widespread usage of biliproteins as fluorescent markers. Much work has gone into developing biliproteins as fluorescent fusion tags because they can absorb light wavelengths that reach the infrared.

Therefore, open-chain tetrapyrroles have applications in many different fields. These include chromophores in genetically modified fluorescence proteins [80], natural food colorants [81], cosmetic patches [82], photosensitizers for photodynamic therapy [83], fluorescent markers [84], absorbing elements in dye-sensitized solar cells [85], and absorbing elements in genetically modified solar cells [83], among many other fields. APCs are the building blocks of PBPs, which are massive light-harvesting antenna complexes found in cyanobacteria and red algae [86]. The α- and β-subunits of APC, ApcA, and ApcB unite to form heterodimers, which autonomously assemble into disk-shaped (αβ)3 trimers. ApcA/ApcB trimers are found in the central cavity of PBPs. The linker proteins ApcE (also known as LCM), ApcD, and ApcF are included in the PBS core. Because APC trimers are naturally luminous, they are frequently utilized as labels in immunofluorescent methods [87]. The conserved Cys residue and the C31 atom of pyrrole ring A are connected via a thioether bond formed by APCs using a PCB chromophore. ApcE binds PCB through an autocatalytic thioether interaction with Cys196. However, bilin lyases are the enzymes that ApcA, -B, -D, and -F require in order to appropriately attach to PBPs to establish a thioether bond with conserved Cys81 residues [88]. Though less effective, the later APCs can bind chromophores without lyases [89]. All APCs have comparable structures: an APC-like domain has seven α-helices that adopt a globin-like fold and an N-terminal extension mostly used in oligomerization [12,90]. In E. coli, APCs are generated as red FPs by the co-expression of PCB synthesizing enzymes and the proper bilin lyases [91]. Notably, APCs’ spectral characteristics may differ based on where they come from. For instance, “ApcD from Nostoc sp. displays a fluorescence peak at 663 nm, but recombinant ApcD from Synechocystis sp. exhibits fluorescence with an emission peak at 642 nm” [88]. With an APC-like domain at its N-terminus, ApcE is a massive membrane-associated protein. Its APC-like domain has a 15% quantum yield and a maximum fluorescence at 672 nm when produced as FP in E. coli. ApcE-based monomeric soluble fluorescent proteins (FP) were produced with a quantum yield of 6% with emission at 663 nm by truncating the hydrophobic loop and N-terminal residues [92]. Trichodesmium erythraeum ApcA was modified to create the far-red FP known as smURFP. TeApcA could bind tetrapyrrole biliverdin (BV) without the need for lyases, thanks to amino acid changes identified through guided mutagenesis. Despite its fluorescence in mammalian cells treated with abundant exogenous BV, smURFP pales compared to BphP-derived NIR fluorescent proteins, including miRFPs [80]. APCs are strongly fluorescent and naturally evolved to maximize fluorescence resonance energy transmission. If everything is considered, APCs encourage molecular templates to create additional red-shifted NIR FPs.

5. Challenges and Future Perspectives in Biomedical Applications

PBPs derived from cyanobacteria possess various biomedical applications, including anti-oxidant, anti-inflammatory, anti-metabolic diseases, anti-cancer, anti-neurodegenerative, anti-pathogenic, etc. [24]. Despite being an important exploitation source in biomedical applications, the major challenge is the production of PBPs on a commercial scale. Extraction and purification at a laboratory scale are possible, but some bottlenecks, including poor selectivity, high energetic costs, and high investment in equipment such as chromatographic techniques, make it difficult to commercialize the production of cyanobacteria PBPs [93]. These drawbacks can be solved through linkages between laboratory researchers and industrial technologists. Although cyanobacteria sustain minimalistic nutrients such as light, water, CO2, and other nutrients, the challenge is to design the industrial bioreactor based on the light exposure and intensity required. A bioreactor should be designed so that the light intensity is uniformly distributed. An adequate amount of light is required for cyanobacteria growth, whereas too high an intensity of light can lead to photoinhibition or
overheating [94]. To utilize biomedical applications and deliver services to a broad population, researchers need to focus on developing various approaches directed toward low-cost production and harvesting technologies. There are few cyanobacteria where the production techniques for PBPs have been developed. It is of utmost importance to develop sustainable production technologies for the mass culture of a broader range of cyanobacteria species. Recently, there has been significant progress in the growth of some cyanobacteria through the use of genetic tools and multiomics approaches. There is a great deal of potential to be further investigated in the near future, thanks to sophisticated genome editing techniques.

6. Conclusions

The review delves into the fascinating realm of cyanobacterial PBPs, shedding light on their intricate structures, diverse functions, and promising biomedical potential. Through a thorough exploration of the existing literature, it becomes evident that these pigmented proteins play a pivotal role in the photosynthetic machinery of cyanobacteria. Their unique spectral properties enable them to efficiently capture light energy across a wide range of wavelengths, facilitating photosynthesis in various environments.

Additionally, their antioxidative properties and potential anti-inflammatory effects suggest their utility in biomedical research and drug development. The biomedical potential of PBPs extends to areas such as cancer therapy, where their ability to selectively target cancer cells through photodynamic therapy holds promise. Moreover, their antioxidant properties could have implications for combating oxidative stress-related diseases. The present work underscores the significance of cyanobacterial PBPs in the natural world and highlights their multifaceted roles and biomedical applications. As our understanding of these remarkable molecules continues to grow, so does the potential for innovative applications in various scientific and medical fields, paving the way for a brighter and more sustainable future.

In conclusion, the radiant world of cyanobacterial PBPs presents a captivating area of research with immense scientific, biotechnological, and biomedical potential. Continued exploration of their structures, functions, and applications not only deepens our understanding of fundamental biological processes but also opens doors to innovative technologies and medical treatments.

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