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Review Article

Current status of pharmacological potentials of phycocyanin

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ABSTRACT

Phycocyanin (PC) is a blue, light harvesting pigment in cyanobacteria, rhodophytes and cryptophytes. It is one of the phycobiliproteins that forms phycobilisome- a light harvesting complex in cyanobacteria. PC is water soluble and strongly fluorescent. It has recently been exploited for its fluorescent and pharmacological properties. Moreover, its use as the healthy ingredient in cyanobacterial based foods and food additive is also gaining recognition due to the increasing awareness of harmful effects of synthetic compounds and inclination of community towards the usage of natural products. More recently, PC content of phytoplankton has proved to be a useful index for the amount of cyanobacteria in water sample. The present review focuses on recent developments in clinical, dietary and fluorescent applications of phycocyanin.

Keywords: Phycocyanin, *Spirulina platensis*, anti-oxidative properties, anti-inflammatory, fluorescent probe, food additive

INTRODUCTION

Phycocyanin (PC) is a blue colored, highly fluorescent, water-soluble light-harvesting pigment found in cyanobacterial species, as well as in eukaryotic chlorophyta, rhodophyta, and bacillariophytaspecies [1]. PC synthesized in *Spirulina* is gaining commercial attention due to its nutrition and healthcare potential. Apart from *S. platensis* many other cyanobacterial species like *Anabaena*, *Galdieria sulphuraria* and other blue-green algae also display high PC contents. PC obtained from *S. platensis* has been widely used as a food additive and cosmetic colorant in Japan [2]. PC and related phycobiliproteins are exploited for its use in cosmetics, biotechnology, diagnostics and medicine. Its use in medicine and biology is attracting increasing attention. Recent studies have demonstrated that PC functions in antioxidation [3], inflammation [4], antitumor [5], and immunity enhancement [6]. There are 55 patents on phycobiliprotein production, 30 patents on its applications in medicine, foods and other areas, and 236 patents on applications utilising the fluorescence properties of phycobiliproteins [7].

Structure and functions: PC along with the other phycobiliproteins- allophycocyanin (APC) and phycoerythrin (PE) comprises a supramolecule known as phycobilisome. The phycobilisome (PBS) is a large, thylakoid membrane associated antenna complex that efficiently absorbs the light between 500–660 nm, a range of visible-light wavelengths not strongly covered by chlorophyll-based antenna complexes [8-10] with their absorption maxima at approximately 540 nm (PE), 620 nm (PC) and 650 nm (APC).The light energy is eventually transferred to the photochemical reaction center of photosystem II (PSII) or photosystem I (PSI) at better than 95 % efficiency [11]. PC is always situated at the ends of the peripheral rods, adjacent to the core cylinders composed of allophycocyanin [12]. It is composed of two relatively homologous subunits- α and β protein subunits of 17 and 19 kDa, respectively, with one phycocyanobilin chromophore attached to cysteine 84 and two phycobilins attached to the β subunit at cysteine 84 and 155 [13]. Moreover, in other phycobiliprotein containing cyanobacteria and microalgae, phycobiliproteins are mobilised as nitrogen storage compounds during periods of nitrogen limitation [14, 15], thereby, playing a secondary role as intracellular nitrogen storage [1].

Anti-oxidant properties: PC can effectively eliminate hydroxyl and oxygen free radicals and possesses antioxidant and anti-inflammatory properties [16, 17]. Free radicals are involved in

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the occurrence of many diseases, including inflammation, atherosclerosis, cancer, reperfusion injury, and other disorders caused by oxidative stress [18]. PC is a potential neuroprotective agent that can be applied to treat oxidative stress-induced neuronal injury in neurodegenerative diseases, such as ischemic stroke, Alzheimer's disease, and Parkinson's disease [19]. Min *et al*. [20] established 2D and 3D astrocyte tissue models to determine the effect of PC on upregulation of antioxidant enzymes [e.g., Superoxide dismutase (SOD), catalase (CAT)], brain-derived neurotrophic factor, relief of inflammation factors [e.g., interleukin-6 (IL-6), interleukin-1β (IL-1β), and glial scar], and improvement of 3D neurons activity. They showed that PC can decrease infarct size and increases behavior disorder in rats with cerebral artery obstruction. Moreover, PC can improve the survival and proliferation ability, weaken the apoptosis of oxidized astrocytes and free radical scavenging ability, and cause no damage to the normal astrocytes and neurons. Mitra *et al*. [21] compared the protective effects of PC and N-acetylcysteine (NAC, a neuroprotective drug) on tributyltin chloride-induced neurotoxicity. They found that both of them can reduce oxidative stress and inflammation, although their mechanisms vary; NAC can effectively regulate enzymes related to the oxidation pathway, whereas PC resists ROS. Another study revealed that PC exerts antioxidant activity by maintaining the activities of cellular antioxidant enzymes, including total glutathione peroxidase (GPx) and selenium containing GPx (GPx-Se), and by increasing reduced glutathione in cells against iron-induced oxidative stress [22]. Madhyastha *et al.*, [23] demonstrated the comparative antioxidant properties of PC from *S. fussiformis* by various antioxidant assay such as 2,2-azinobis-ethylbenzthaiazoline-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH). The observed antioxidant activities were compared with that of ascorbic acid, a standard antioxidant. Benedetti *et al*. [24] showed the efficacy of a novel natural extract from *Aphanizomenon flos-aquae* enriched with PC in protecting human erythrocytes and plasma samples against the oxidative damage induced by 2,2V-Azobis (2-amidinopropane) dihydrochloride (AAPH). Wang *et al.*, [25] demonstrated that recombinant PC/β from *Anabaena* PCC 7120 can inhibit cell proliferation and induced apoptosis by promoting cytoskeleton depolymerization and activating the caspase activities that are associated with the extrinsic cell death pathway, suggesting the antioxidant potential of PC. Roy *et al.*, [26] demonstrated that PC can scavenge reactive oxygen species (ROS) and reduced drug resistance in cancer cell. Moreover, hepatoprotective effects of PC are widely researched and documented [27, 28]. PC can inhibit the activities of antioxidant enzymes, GPx, glutathione reductase, glutathione-S-transferase, and CAT and reduce renal toxicity [29-31]. Zhou *et al*. [32] examined how different factors affect the antioxidant activity of phycocyanin. While phycocyanin generated hydroxyl radicals in the light, it scavenged them in the dark. Moreover, phycocyanin lost its ability to generate hydroxyl radicals when denatured but became much more efficient at scavenging them.

Table. 1 Antioxidant activity of phycocyanin

Antioxidant effect of PC	**Experimental systems**	**References**
Inhibits methyl linoleate peroxidation and oxidation of phosphatidylcholine liposomes	*Spirulina platensis*	[33]
Overproduces of intracellular ROS and MDA and causes changes in SOD and GSH-Px enzymic activities	Human	[34]
Scavenges peroxyl and hydroxyl radicals	*S. platensis*	[35]
Normalizes urinary and renal oxidative stress markers, and expression of NADPH oxidase component	Mice	[30]
Reduces oxidative stress and apoptotic markers, astroglial activation and cell death	Rat	[21]
Prevents cholesterol- induced artherosclerosis	Hamster	[36]
Prevents oxalic acid-induced kidney stone formation by preventing lipid peoxidation	Rat	[37]
Prevents thioacetamide-induced hepatic encephalopathy by preventing lipid peoxidation	Rat	[38]
Reduces cardiotoxicity of the drug doxorubicin by scavenging oxygen radicals	Rat cardiomyocetes	[39]
Causes anti-platelet aggregation due to inhibition of cyclooxygenase	Rabbit plasma	[40]
Induces apoptosis in human hepatocellular- carcinoma cells	Human	[26]
Inhibits proliferation of human myeloid leukemia cells	Human	[41]

Induces apoptosis in human myeloid leukemia cells	Human	[42]
Decreased infarct size and increases behavior disorder in rats with cerebral artery obstruction	Rat	[19]
Affects on astrocytes-mediated neuroprotection against oxidative brain injury	Rat	[20]
Reduces phagocytosis and the associated respiratory burst activity in Kupffer cells	Mice	[43]
Reduces carbon tetrachloride induced hepatocyte damage *in vitro* and *in vivo*	Human	[28]
Prevents cisplatin-induced nephrotoxicity through inhibition of oxidative stress	Mice	[27], [31]
Protects against diabetic nephropathy by inhibiting oxidative stress	Mice	[30]
Reduces sodium-selenite induced cataract	Rat	[44],[45], [46]
Protection of lens epithelial cells	Human	[47]
Reduces serum cholesterol, total cholesterol triglyceride and low density lipoprotein	Hamster	[48]
Inhibits progress of atherosclerosis by reducing blood fat levels	Mice	[49]
Protects from Tributyltin (TBT) induced thymic atrophy by its radical scavenging properties	Rat	[18]

Anti-inflammatory properties: Romay *et al*. [17] showed for the first time that PC inhibited peroxide induced paw edema in mouse in a dose dependent fashion. Reddy *et al*. [50] showed that PC, being a selective cyclooxygenase-2 (COX-2) inhibitor, displays certain hepatoprotective, anti-inflammatory, and anti-arthritic properties. COX-2 is the key enzyme involved in the biosynthesis of prostaglandins (PGs) which play an important role in inflammation, pain and variety of other disorders. PC has a very promising anti-inflammatory effect on colitis and osteoarthritis. The anti-inflammatory effect of PC was first reported in experimental model of colitis in rats [51]. The evidence for anti-arthritic properties of PC of *S. platensis* in collagen induced arthritis was given by Kumar *et al*. [52] in female wistar rats. More recently, Martinez *et al.* [53] showed that the dietary supplement containing PC reduced various inflammatory cytokines, such as TNF-*α*, IL-6, MMP-3, NO, and sulfated glycosaminoglycans as treatment of osteoarthritis. Chen *et al.* [54] reported that PC can inhibit the expression of inflammation-related genesin LPS-stimulated BV-2 microglial cells by downregulating the RNA expression levels of inducible NO synthase (iNOS), COX-2, TNF-*α*, and IL-6 and release of lactate dehydrogenase. Shih *et al.* [55] also displayed anti-inflammatory potential of PC. They observed that PC inhibited the overexpression of NO and PGE2 by downregulating the expression of iNOS and COX-2 and reducing the formation of TNF-*α* and the infiltration of neutrophils into inflammation sites in rats.

Immunomodulatory properties: The aqueous extract of *Spirulina* was found to have a major impact on the immune system by increasing the phagocytic activity of macrophages, stimulating the NK cells. It also played a role in the activation and mobilization of T and B cells due to its stimulatory effects in the production of cytokines and antibodies [56]. Thus, PC plays an important role in modulating the immune system. PC has been reported to suppress the growth of tumor cells, to promote NK cell activity and to induce the lymphocytes in spleen to produce TNF-α in *in-vivo* and *in-vitro*. PC also had an inhibitory effect on the release of histamine from mast cells during an allergic inflammatory response [42]. Hayashi *et al*. [57] reported that PC enhanced the biological defense activity in C3H/ HeN and BALB/cA mice by reducing allergic inflammation by suppressing the antigen specific IgE antibody and through maintaining the mucosal immune system functional against infectious diseases. PC can also improve erythropoietin activity of cells and then directly stimulate the formation of colony forming unit-erythroid, which will stimulate bone marrow hematopoiesis [58]. Peng *et al*. [59] showed that PC can enhance the activity of lymphocytes, the immunity of an organism, and the body's ability to prevent and resist disease. Studies by Zhou *et al*. [60] confirmed that PC can promote phytohemagglutinin stimulated lymphocyte transformation, recover the E-rosette forming ability of T cell after damaging by cyclophosphamide, and significantly improve the number of antibody forming cells and their abilities to produce antibodies in normal rats and immune hypofunction mouse spleen cells treated with

hydrocortisone. A study of PC-mediated photodynamic therapy (PDT) on rat tumor model and *in vivo* and *in vitro* apoptosis mechanism of MCF-7 cells showed that PC can enhance the proliferation of immune organs and immune cells indicating that PC can promote immune function and resist diseases [61]. In addition, Nemoto-Kawamura *et al*. [62] showed that PC can prevent or downgrade experimental autoimmune encephalitis (EAE) expression and upregulate the expression of key markers for regulatory T cell (Treg): fork head protein 3, CD25, IL-10, and TGF-β when used to treat EAE. In addition, PC might act as a neuroprotector that reverses damage in neurodegenerative disorders of the central nervous system, thereby improving the myelin and axonal damage of EAE. Thus, PC demonstrates a therapeutic potential for multiple sclerosis and may lead to effective therapies by activating Treg [63].

Table.2 Immunomodulatory and anti-inflammatory activities of phycocyanin

Anti-inflammatory and immunomodulatory properties of PC	**Experimental systems**	**References**
Reduced expression of iNOS, COX-2, TNF-α and IL-6 mRNA	Murine BV-2 microglial cells	[54]
Induced secretion of TNF-α, IL-1β, and IL-6; increased expression of pro-IL-1β and COX-2 protein	murine macrophage cell line J774A.1	[64]
Decreased IL-4 production and increased IFN-γ production	Human blood mononuclear cells	[65]
Increased production of SOD, NF-κβ	Nonalcoholic steatohepatitis model rat	[66]
Reduces induced MPO activity; inhibition in inflammatory cell infiltration and reduction to some extent in colonic damage	Rat	[51]
Downregulates mRNA expression of NR2B, TNF-α, IL-1β and COX-2 genes in the ear cochlea	Mice treated with salicylate	[67]
Inhibits COX-2 with an IC_{50} value of 80 nM	Human whole blood	[50]
PC based nutritional supplement reduced various inflammatory cytokines such as TNF-α, IL-6, MMP-3 and NO as a treatment to osteoarthritis and collagen induced arthritis	Canine chondrocytes Rat	[53] [52]
Inhibits overexpression of NO and PGE2 by downregulating the expression of iNOS and COX-2	Rat Murine macrophage cell line	[55] [68]

Florescent properties: Phycobiliproteins are brilliantly colored and highly fluorescent pigments. The colors of the phycobiliproteins arise from the presence of chromophores- bilins, which are covalently attached to cysteine residues of the apoproteins. The bilins are linear or open-chain tetrapyrroles derived biosynthetically from heme via biliverdin. The visible absorption spectra of individual phycobiliproteins arise from the particular bilins attached to the protein and modulated by the conformation, environment and interchromophore interactions [69]. When phycobilisomes are extracted into aqueous buffers, they disintegrate and the phycobiliproteins lose their natural acceptors of excitation energy and become highly fluorescent. Compared to other fluorophores, phycobiliproteins have high molar extinction coefficients, fluorescence quantum yields, and large stokes shifts [1]. The phycobiliproteins serve as valuable fluorescent tags with numerous applications in flow cytometry, fluorescence activated cell sorting, histochemistry, and to a limited degree in immunoassay and detection of ROS exploiting the unique physical and spectroscopic properties of phycobiliproteins [69]. The phycobiliproteins were introduced as a novel class of fluorescent dyes in 1982 [70]. PC is widely commercialized for fluorescent application in clinical and immunological analysis used in diagnostic assays and in diverse research applications [71, 72].

Table.3 Applications of flourescent properties of phycocyanin

Applications of fluorescent properties of phycocyanin	**References**
Used as fluorescent dye for medical labeling	[73]
Used in monitoring of potentially harmful cyanobacterial populations in local habitats	[74],[75], [76], [77], [78], [79], [80]
Used in detection of toxic cyanobacteria in drinking water	[81]
Used as biospecific fluorescent probe. Genetically stabilized phycocyanin fusion proteins fused to biospecific fluorescent probe	[82]
Used as fluorescence emitter in fluorescence-activated cell sorting flow cytometry	[99]

Food additives and natural dye: Due to the toxic effect of several synthetic dyes, there is an increasing demand for natural colorants in food, pharmaceuticals, cosmetics, textile and printing industries. Phycobiliproteins, specifically PC and PE are used as a natural protein dye in the food and cosmetic industries [85]. The common method for human intake of PC is in a non-purified form via *Arthrospira platensis* health food products [86]. The Japanese Company Dai Nippon Ink and Chemical Company extracts the blue PC from *A. platensis* and sells it as a natural blue pigment for use in health foods and cosmetic products [87]. Furthermore, PC is also used in the coloring of many food products such as fermented milk products, ice creams, soft drinks, desserts, sweet cake decoration, milk shakes, chewing gum and jellies [88].

A few studies have addressed the functionality of PC in foods with regard to color stability [89] and rheological properties [90]. Sekar & Chandramohan, [7] reported that although PC is less stable in heat and light, it is considered more versatile than gardenia and indigo, showing a bright blue color in jelly, gum and coated soft candies. It was found that the blue pigment produced from *Phorphyridium aerugineum* does not change with pH and was stable under light, however, it was found to be sensitive to heat. Within a pH range of 4 to 5, the blue color produced is stable at 60°C for 40 minutes [91]. 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 (Pepsi® and Bacardi Brezzer®) which did not lose their color for at least 1 month at room temperature. The color was stable in dry preparations such as sugar flowers for cake decorations which maintained their color for several years of storage.

CONCLUSION

PC has gained a lot of commercial interest due to its fluorescent, nutraceutical and pharmacological properties. Studies on animal model as well as human suggest the positive health effect of dietary supplements rich in PC or intake of purified PC. It has strong anti-oxidant anti-inflammatory, neuroprotective and hepatoprotective properties which make PC a strong contender as a nutraceutical or pharmaceutical agent. Increasing side effects due to toxic synthetic food colorants have increased the need for a nontoxic food additive. The intense cyan or blue color of the pigment has made it popular in the food industry. It is widely used in food jellies, ice-creams and candies as well as cosmetic industries. The strong fluorescence nature of this pigment has been exploited for its use as a diagnostic probe and in on-line monitoring of phytoplankton in water reservoirs.

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