Bio-regulatory Functions of Biliproteins and Phycobilins from Algae

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SUMMARY: Rhodophyta, Cyanobacteria or other algae contain phycobiliproteins as light-harvesting components for photosynthesis. The bio-regulatory functions of the proteins or their chromophores are introduced. It has been shown that both proteins and chromophores effectively quench various active oxygen species *in vivo* and *in vitro*. Phycoerythrobilin and phycocyanobilin, corresponding to the chromophores of phycoerythrin and phycocyanin, respectively, were mainly responsible for antioxidant activities of the chromoproteins while phycocyanin has anti-inflammatory activity partly due to its specific cyclooxygenase 2 inhibitory property of its apoprotein. Phycocyanobilin also suppressed platelet aggregation induced by both collagen and ADP.

KEY WORDS: oxidation, inflammation, platelet aggregation, phycocyanin, phycoerythrin

INTRODUCTION

Phycobiliproteins are light-harvesting components for photosynthesis in cyanobacteria, Rhodophyta, Cryptophyta and etc. Phycobiliproteins contain one or more covalently linked linear tetrapyrrole chromophores, which give intensely blue, red, blue-green or violet-blue color to the proteins. Despite the fact that algae containing these proteins have been consumed as nutritious foods in various countries, there is very limited information available on the biological functions of the proteins or the chromophores in our body.

Stocker *et al.*¹⁾, reported that bilirubin in human plasma is an effective scavenger for various reactive oxygen species *in vivo*. The bio-regulatory functions of the proteins or the chromophores have become study subjects attracting the interest of many researchers in these years since phycocyanobilin in phycocyanin is known to have a structure similar to that of bilirubin.

In this paper, we summarized these functions and introduced some of them mainly on the basis of the results obtained by our investigation.

SUMMARY OF FUNCTIONS

The bio-modulating functions of biliproteins and phycobilins including antioxidative, anti-platelet aggregative, anti-neuronal damage, hepatoprotective and anti-inflammatory activities are listed in Table 1. The proteins and their chromophores show multifunctional features mainly based on their antioxidative activities. Functions of phycoerythrin have not been studied well compared with those of phycocyanin.

ANTIOXIDATIVE ACTIVITY

Fig. 1 shows typical antioxidative activities of the proteins. When the formation of linoleic acid peroxides was induced in the phosphate buffer, both R-phycoerythrin and C-phycocyanin effectively inhibited the peroxidation of linoleic acid and produced the prolonged induction periods. The rates of the peroxides formation were proportional to the decreasing rates of absorbance in visible region of each chromoprotein²⁾.

Fig.1 Time-dependent oxidation of linoleic acid with and without biliproteins. R-phycoerythrin (PE) and C-phycocyanin (PC) were extracted from laver, *Porphyra* sp. Linoleic acid with/without PE or PC in a phosphate buffer was oxidized by 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH). Linoleic acid hydroperoxides formed were periodically determined by HPLC.

Antioxidative activities of phycocyanobilin as well as various phytochemicals were also evaluated in the phosphate buffer (Fig.2)³. In the reaction mixture without phycocyanobilin, the oxidation of linoleic acid resulted in a linear accumulation of hydroperoxides of the acid while phycocyanobilin effectively inhibited the peroxidation of linoleic acid. Among the chemicals examined, quercetin showed the most potent antioxidative activity. These flavonoids and phenolic carboxylic acids have recently attracted the attention of many researchers. Caffeic acid has a marked radical scavenging activity among other hydroxycinnamic acids such as *p*-coumaric acid and ferulic acid.⁴ A typical flavonol quercetin also act as an effective scavenger for active oxygen species, especially when they attack plasma lipoproteins and biomembranes⁵. Other phytochemicals except genistein also exhibit the same levels of antioxidative activities. These results indicated that phycocyanobilin has the antioxidative activity equal to those of the flavonoids and phenolic carboxylic acids¹⁸, especially in the early stage of oxidation.

These and other experiments carried out by other groups shown in Table 1 suggested that both biliproteins and phycobilins have effective antioxidant ac-

Fig.2 Antioxidative activities of phycocyanobilin (PCB) and several phytochemicals and change in absorbance of phycocyanobilin at 610nm. Linoleic acid with each compound was oxidized by AAPH, and hydroperoxides formed were determined by HPLC. Each value shows the average from two measurements. ●:control, ■:PCB (10 μ M), □:catechin (10 μ M), ▲:quercetin (10 μ M), △:caffeic acid (10 μ M), ×:chlorogenic acid (10 μ M), ○:genistein (10 μ M), ◆: absorbance of PCB at 610nm.

Table 1 Functions of phycobiliproteins and phycobilins

Functions	Inducer	Target	Inhibitor	Ref.
Antioxidative	O ₂ ⁻ (Xanthine oxidase+Hx)	Luminol, 2-Deoxyribose	PC	6
	RO $\cdot$ (t-BOOH)			
	OH $\cdot$ (Fenton's reaction)			
	AAPH	Linoleic acid, PC liposome	PC, PE	2
		Linoleic acid	PCB	3
		Human LDL and Chol in plasm	PCB	7
		PC liposome	PCB	8
		Direct reaction with PC		9
	AMVN $\cdot$	Methyl linoleate	PCB	8
	Cu ²⁺	Human plasma	PCB	7
	Leucocyte	Luminol	PC	6
	Fe ²⁺ , Ascorbic acid	Liver microsomal lipid	PC	6
	Singlet oxygen	Methyl linoleate	PCB	7
	Soybean lipoxidase	Linoleic acid	PCB, PEB	7
	HOCl	Direct reaction with PC		10
Anti-neuronal damage	Kainic acid	Hippocampus	PC	11
Hepatoprotective	CCl ₄ , Pulgone	Rat	PC	12
Anti-inflammatory	Glucose oxidase, AA,	Mouse paw, ear, axilla	PC	6,13,14,15
	TPA, Carageenan, Cotton			
	pellet			
	Cyclooxygenase I, II	Arachidonic acid	PC	16
Anti-platelet aggregative	Acetic acid	Rat colonic tissue	PC	17
	ADP, Collagen	Human platelet	PCB	This work

PC:C-Phycocyanin, PCB:Phycocyanobilin, PE:R-Phycocerythrin, PEB:Phycocerythrobilin

tivities *in vivo* as well as *in vitro*. However, the active sites and the antioxidative mechanisms are unknown. Some amino acid residues in a polypeptide chain are known to exhibit antioxidant activities. Wayner *et al.*,¹⁹ for example, suggested that human serum albumin highly contributes to antioxidation in the plasma, and sulfhydryl groups are responsible for the activity. Since apophycocyanins prepared from various cyanobacteria contain these antioxidant amino acid residues,²⁰ a possible contribution of the apoprotein to the total antioxidant activity of C-phycocyanin cannot be excluded. A remarkable antioxidative activity of phycocyanobilin obtained in Fig.2, however, suggested that the tetrapyrrol chromophore is highly responsible for the activity of biliproteins. The absorbance of phycocyanobilin (610nm) rapidly decreased during the induction period, indicating the occurrence of cleavage of conjugated double bonds in the chromophore (Fig.2, dashed line). The possible cleavage of conjugated double bonds in its tetrapyrrol structure may indicate involvement of a radical addition reaction to the double bonds.

When concentrations of C-phycocyanin and phycocyanobilin in the reaction mixture were equally adjusted on the phycocyanobilin basis, and antioxidant activities of both chemicals were evaluated after adding AAPH, the activity of phycocyanobilin was almost the same as that of C-phycocyanin.⁸⁾

These results also support an idea that the antioxidant activity of C-phycocyanin is attributable to phycocyanobilin, chromophore in C-phycocyanin.

ANTI-INFLAMMATORY ACTIVITY

Recently, anti-inflammatory activity of C-phycocyanin induced by glucose oxidase was reported.⁶⁾ C-phycocyanin was orally administered to OF1 mice and one hour later glucose oxidase was injected into their hind paws. When weight differences between left and right paws were determined as edema index, C-phycocyanin effectively inhibited peroxide-induced inflammation in a dose dependent manner. Glucose oxidase generates hydrogen peroxide and subsequently hydroxyl radical. Therefore C-phycocyanin effect must be due at least in part to the scavenging of hydroxyl radical. On the other hand, this effect can be explained from the viewpoint of an inhibitory activity of C-phycocyanin against cyclooxygenase 2¹⁶⁾. IC₅₀ of C-phycocyanin against cyclooxygenase 2 activity was ca.0.18 μ M which is very effective even when compared with that of Rofecoxib known as a specific cyclooxygenase 2 inhibitor. When C-phycocyanin was reduced, the inhibitory effect significantly decreased. This suggests that the

protein structure is primarily responsible for the inhibitory activity by cyclooxygenase 2. It is generally known that lipid peroxidation induced by free radicals activate cyclooxygenase resulting in the formation PGs from arachidonic acid. Therefore, direct inhibition of cyclooxygenase 2 by C-phycocyanin may lead to suppression of inflammation induced by the *in vivo* experiment. C-phycocyanin is also effective to suppress other types of inflammation (Table 1).

ANTI-PLATELET AGGREGATIVE ACTIVITY

Fig.3 shows anti-platelet aggregative activity of phycocyanobilin. Fifty μ M phycocyanobilin effectively control the aggregation although the effect is not drastic. Various binding sites on platelet surface activate to aggregate after calcium ion is mobilized from dense tubular system in platelet. Fibrinogen is a representative mediator to combine each platelet.

Therefore if calcium mobilization is determined, we can estimate either phycocyanobilin affects pre-mobilization or post-mobilization of calcium. About 20% mobilization was suppressed at the phycocyanobilin concentration (50 μ M) for 50% inhibition of platelet aggregation (Fig.4). This level of suppression for calcium mobilization does not completely explain 50% inhibition of the aggregation. However at least in part phycocyanobilin affects pre-mobilization system of calcium. C-phycocyanin may be also effective to control platelet aggregation since C-phycocyanin suppresses cyclooxygenase activity, and the mechanism of platelet aggregation

Fig.3 Platelet Aggregation Induced by ADP and Collagen. Human platelet was freshly collected and activated by ADP and collagen. Aggregation was determined with Hema Tracer 212 (MCM medical).

Fig.4 Relative mobilization of calcium ion from dense tubular system in platelet. Calcium in platelet was fluorometrically determined²¹⁾.

involves cyclooxygenase reactions and PGs productions from arachidonic acid.

CONCLUSION

Bio-regulatory functions, recently, has become the center of wide interest. Aquatic products including algae must be inexhaustible resources containing "functions". Further studies are required to screen and clarify functional components, and to develop food rich in "functions".

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