Inflamm. res. 47 (1998) 36–41
1023-3830/98/010036-06 $ 1.50+0.20/0

Inflammation Research

Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae

C. Romay[1], J. Armesto[1], D. Remirez[1], R. González[1], N. Ledon[1] and I. García[2]

[1]Pharmacology Department, National Center for Scientific Research, CNIC, P.O. Box 6990, Havana, Cuba, Fax 53 7 330497, e-mail: ricardo@quimica.cneuro.cu
[2]Pharmacy and Foods Faculty, University of Havana, Ave. 23, No. 21425, La Coronela, Havana, Cuba

Received 21 April 1997; returned for revision 14 May 1997; accepted by M. J. Parnham 28 October 1997

Abstract. *Objective:* Phycocyanin is a pigment found in blue-green algae which contains open chain tetrapyrroles with possible scavenging properties. We have studied its antioxidant properties.
Materials and methods: Phycocyanin was evaluated as a putative antioxidant in vitro by using: a) luminol-enhanced chemiluminescence (LCL) generated by three different radical species (O_2^-, $OH^\bullet$, $RO^\bullet$) and by zymosan activated human polymorphonuclear leukocytes (PMNLs), b) deoxyribose assay and c) inhibition of liver microsomal lipid peroxidation induced by Fe^{+2}-ascorbic acid. The antioxidant activity was also assayed in vivo in glucose oxidase (GO)-induced inflammation in mouse paw.
Results: The results indicated that phycocyanin is able to scavenge $OH^\bullet$ ($IC_{50} = 0.91$ mg/mL) and $RO^\bullet$ ($IC_{50} = 76\,\mu$g/mL) radicals, with activity equivalent to 0.125 mg/mL of dimethyl sulphoxide (DMSO) and 0.038 μg/mL of trolox, specific scavengers of those radicals respectively. In the deoxyribose assay the second-order rate constant was $3.56 \times 10^{11}\ M^{-1}\ S^{-1}$, similar to that obtained for some non-steroidal anti-inflammatory drugs. Phycocyanin also inhibits liver microsomal lipid peroxidation ($IC_{50} = 12$ mg/mL), the CL response of PMNLs ($p < 0.05$) as well as the edema index in GO-induced inflammation in mouse paw ($p < 0.05$).
Conclusions: To our knowledge this is the first report of the antioxidant and anti-inflammatory properties of c-phycocyanin.

Key words: Antioxidant – Chemiluminescence – C-Phycocyanin – Blue-green algae – Free radical scavenger

Introduction

C-phycocyanin is a protein-bound pigment found in blue-green algae. Phycocyanin monomers are themselves made up of two distinguishable protein subunits designated α and β, which contain at least three covalently attached bilin chromophores, open chain tetrapyrroles with no metal complexes [1]. These prosthetic groups account for about 4% of the algae mass, indicating the presence of about sixteen chromophoric groups per unit molecular weight [2]. It occurs in four different structural forms, monomeric, trimeric, hexameric and decameric [3], and is the most abundant pigment in blue-green algae, accounting for more than 20% of algal dry weight [4].

The chemical structure of the bilin chromophores in c-phycocyanin, (open chain tetrapyrroles) are very close to that of bilirubin. Stocker et al. [5] reported that bilirubin is an antioxidant of possible physiological importance because it could scavenge peroxy radicals by donating a hydrogen atom attached to the C-10 bridge of the tetrapyrrole molecule to form a carbon-centered radical with resonance stabilization extending over the entire bilirubin molecule.

It is well known that reactive oxygen species (ROS) are involved in a diversity of important processes in medicine including, among others: inflammation, atherosclerosis, cancer, reperfusion injury [6]. One way by which a substance can interfere with these processes is by acting as antioxidant or free radical scavenger.

Taking these data into account, we postulated that c-phycocyanin may be a putative antioxidant and decided to evaluate it in some in vitro and in vivo experimental models.

Materials and methods

Reagents

Luminol and xanthine oxidase (XO 20 U/mL) were obtained from Boehringer Mannheim GmbH (Germany). Trolox and p-iodophenol were from Aldrich Chemical Co. (Milwaukee, WI, USA). Superoxide dismutase (SOD) (3333 U/mL) from bovine erythrocytes and Hypoxanthine (HX) were from Serva Feinbiochimica (Heidelberg, Germany). Tert-butylhydroperoxide (*t*-BOOH) and butyl hydroxytoluene (BHT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sodium dodecylsulphate (SDS), hydrogen peroxide (H_2O_2), ascorbic

Correspondence to: C. Romay

acid, 2-deoxyribose and thiobarbituric acid (TBA), were from BDH Ltd (Poole, UK). $FeCl_3$ and glucose oxidase (GO) (1.4 U/mg) were from Merck (Darmstadt, Germany). All other reagents were of analytical grade.

Phycocyanin was obtained from Arthospira maxima species and purified by the method of Neufeld and Riggs [7].

Phosphate-buffered saline solution (PBS) consisted of NaCl 0.14 M, KCl 2.7 mM, Na_2HPO_4 12 mM, KH_2PO_4 1.5 mM, CaCl 0.9 mM and $MgCl_2$ 0.49 mM.

Animals

Male OF_1 mice weighing 22–25 g and male Sprague Dawley rats (220–250 g) were purchased from the National Center for Laboratory Animals Production (CENPALAB, Havana, Cuba). The animals were housed under controlled temperature ($t = 25\,^{\circ}C$) and air humidity (60%) with a 12 h light-dark cycle, and kept on a standard laboratory diet and drinking water ad libitum.

Chemiluminescence measurements

The scavenging action of c-phycocyanin was determined against different types of oxygen radicals, which were generated by specific chemical reactions and detected by LCL.

A well known scavenger for each radical was used as control for the paradigm and to compare its effect with that produced by c-phycocyanin.

Chemiluminescence was measured in millivolts in an LKB Wallac 1250 luminometer coupled to an LKB 2210 two channel recorder.

Superoxide radical scavenging activity was determined as described by Pascual et al. [8]. The reaction consisted of 800 μL of a mixture containing 68 mM glycine buffer pH 8.6, 10 μM luminol and 5 μM p-iodophenol. Fifty μL of distilled water or c-phycocyanin aqueous solutions were added. Then 2 μL of xanthine oxidase (2 U/mL) were added and the reaction was initiated with 10 μL of hypoxanthine 1 mM. The intensity was registered immediately. SOD was used in the system as a control.

Hydroxyl radicals were generated from the Fenton reaction. The method used was described by Pascual and Romay [9]. In brief, to 800 μL of a mixture containing: 50 mM K_2HPO_4-KH_2PO_4 buffer pH 7.8, 2 mM EDTA and 0.1 mM luminol, 0.1 mL of distilled water, sample or specific scavenger (DMSO) was added. Then 5 μL of 6 mM H_2O_2 was also added and mixed. The reaction was started with 10 μL of 20 mM $FeSO_4$ and after rapid mixing, the CL signal was immediately registered.

Determination of alkoxyl radical scavenging activity was performed by measuring the inhibition of the CL produced by the reaction of t-BOOH with ferrous ions in the presence of luminol, as previously described [9]. The reaction mixture consisted of: 800 μL of 50 mM glycine buffer pH 8.6, 50 mM SDS, 0.025 mM luminol. Ten μL of distilled water was added and mixed prior to the addition and mixing of 5 μL of 7.3 mM t-BOOH.

The reaction was started with 10 μL of 0.4 mM $FeSO_4$ and immediately after rapid mixing the chemiluminescence signal (mV) was recorded. Trolox, a water soluble analogue of vitamin E, was used as specific scavenger of these radicals.

Double quenching experiments were done in each CL system in order to determine whether the effect of the phycocyanin was due to the scavenging of the desired oxygen free radical or the trapping of other free radical species. These experiments were done by measuring the luminous signal before (Io) and after (I) adding increasing concentrations of c-phycocyanin in the absence and presence (sufficient to cause 50% inhibition) of the specific scavenger (SOD, DMSO or trolox). The slope of the plot is equal to $\kappa\tau$ where τ is the life time of the radical in the absence of phycocyanin. When both phycocyanin and the specific scavenger compete for the same radical, a decrease in the slope must be expected.

Effect of phycocyanin on the CL response of isolated PMNLs

PMNL preparation

Human leukocytes were obtained as described previously [8] from 10 mL heparinized (20 U/mL) venous blood from healthy volunteers, who had not taken any drug during the week before blood sampling. The blood was mixed with an equal volume of an ACD-dextran-glucose mixture consisting of: 1.5 mL acid citrate dextrose (ACD) in 0.9% NaCl (24.5 g glucose/L, 22 g sodium citrate dihydrate/L and 7.3 g citric acid/L), 5 mL of 6% (w/v) Dextran T-500 in 0.9% of NaCl and 3.5 mL of 5% glucose in 0.9% NaCl.

After mixing well and allowing to stand for 45–60 min at room temperature, the upper layer containing the leukocytes was removed by aspiration and three times its volume of ammonium chloride 0.8% added in order to hemolyze the remaining red cells. The cells were centrifuged for 10 min at 500 g at 4 °C. Then, 2 mL of 0.9% NaCl were added, followed by 3 mL of cold distilled water which were mixed and allowed to stand for 2 min, then mixed with 3.6% NaCl and centrifuged for 10 min at 500 g. Finally the cells were resuspended in approximately 5×10^6 cells per mL PBS.

Opsonisation of particles

Cells from a fresh culture of Saccharomyces cerevisiae were washed and then put into a boiling water bath for 30 min. After washing in saline they were resuspended at a concentration of 2×10^8 particles/mL PBS. The opsonisation procedure was carried out by incubating a mixture of 200 μL human serum with 1.8 mL of yeast suspension for 30 min at 30 °C immediately prior to the experiment. A luminol stock solution of 10^{-2} M in DMSO was prepared and was diluted to 10^{-4} M in PBS prior to use.

Chemiluminescence assay

Chemiluminescence was performed as described previously [8, 10] with minor modifications. Briefly, 200 μL of opsonised yeast cells, 450 μL of phosphate-buffered saline (PBS), 200 μL luminol 10^{-4}M and 50 μL of water or different phycocyanin concentrations, were incubated for 10 min in the measuring cuvette. Just before the assay, 100 μL of an isolated leukocyte suspension were added and the light intensity was measured every 3 min at 37 °C. In a system without cells there was no interaction between luminol and phycocyanin. The viability of PMNLs after being exposed to the higher c-phycocyanin concentration (incubated at 37 °C water bath for 40 min) was assessed by the Trypan blue exclusion test. The viability obtained was 98%.

Inhibition of damage to 2-deoxyribose

Evaluation of the inhibition of damage to 2-deoxyribose, measured as formation of thiobarbituric acid-reactive material [11], was carried out as an alternative measure of the hydroxyl radical scavenger capacity of c-phycocyanin. Mixtures contained, in a final volume of 1.2 mL: deoxyribose 2.8 mM, KH_2PO_4/KOH buffer 15 mM pH 7.4, $FeCl_3$ 20 μM, EDTA 100 μM, H_2O_2 2.8 mM and ascorbic acid 100 μM. $FeCl_3$ and ascorbate solutions were made up in bidistilled water just before use. $FeCl_3$ and EDTA were premixed prior to addition to the reaction mixture. Ascorbic acid was added in order to start the reaction. Reaction mixtures were incubated at 37 °C for 1 h. After addition of 1 mL of TBA 1% (w/v) in 0.05 mM NaOH and 1 mL of TCA 2.8% in water, the mixture was heated at 100 °C for 20 min. The pink chromogen that progressively developed was then measured at 532 nm after cooling, against appropriate blanks. The second-order rate constants k_s were calculated using the data obtained in the presence of EDTA from the slope of a plot of $1/A_{532nm}$ against the test compound concentration.

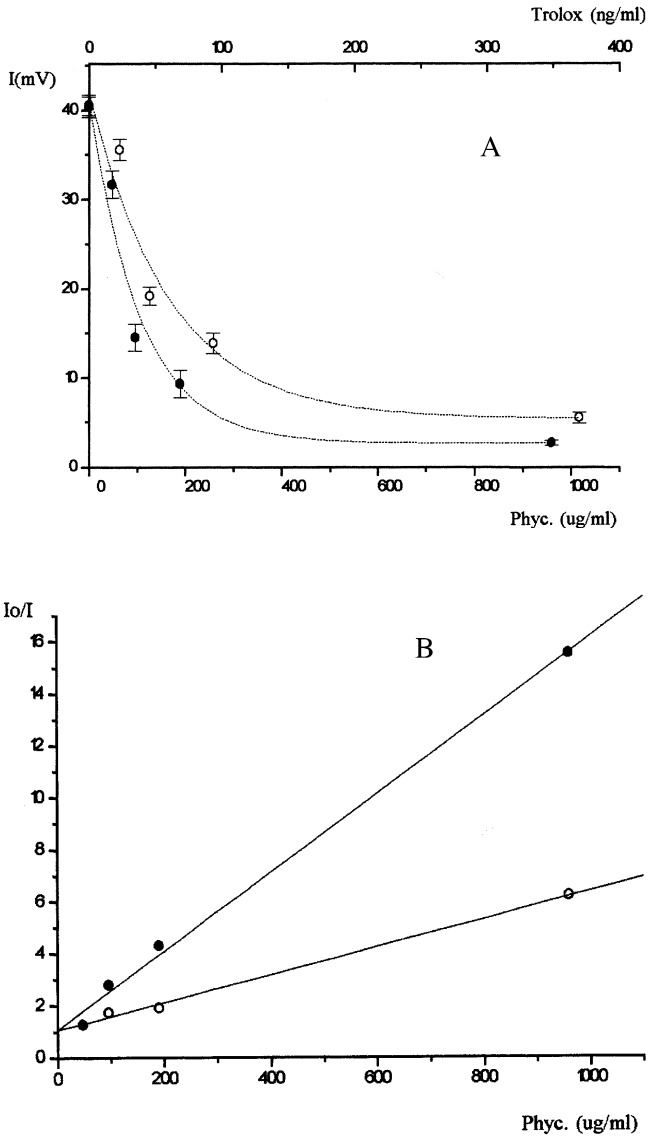

Fig. 1. Effect of c-phycocyanin on chemiluminescence intensity produced by alkoxy radicals generated by the reaction of tert-butylhydroperoxide with ferrous ion. (A) Decrease in Chemiluminescence signal (mV) with increasing c-phycocyanin (●) or trolox (○) concentration. Each point represents the mean of three determinations. Vertical bars represent ± 1 SD. (B) Double quenching experiment carried out to determine the chemiluminescence signal before (Io) and after (I) adding increasing concentrations of c-phycocyanin in the absence (●) and presence (○) of trolox (46 ng/mL). Each point represents the mean Io/I value obtained from three determinations.

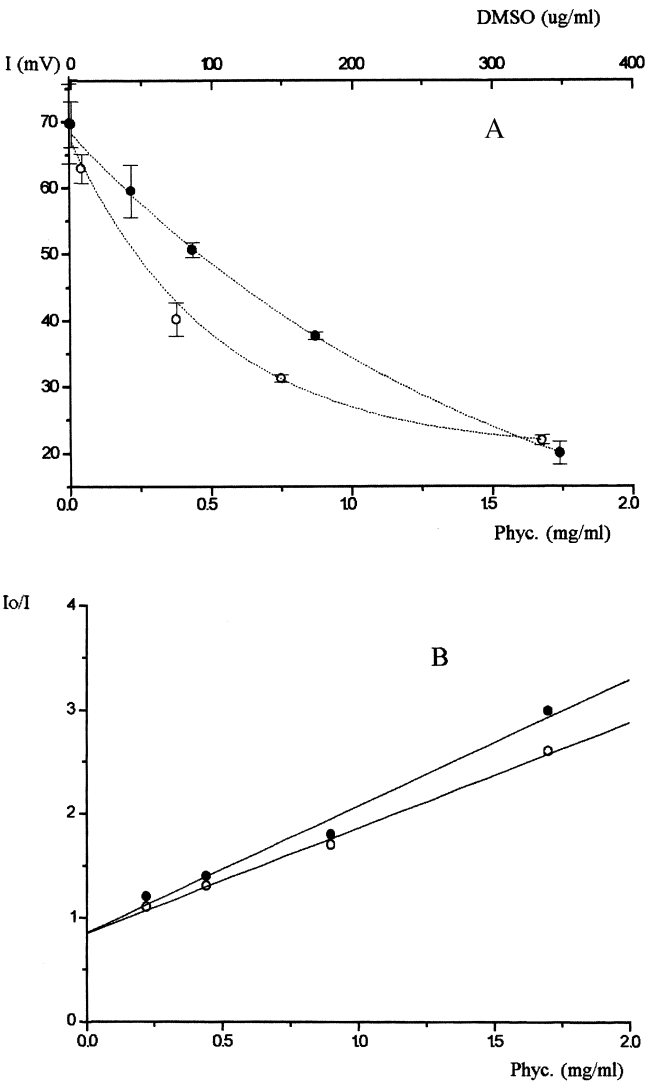

Fig. 2. Effect of c-phycocyanin as scavenger of hydroxyl radicals produced by the Fenton reaction. (A) Decrease in chemiluminescence signal (mV) with increasing c-phycocyanin (●) or DMSO (○) concentrations. Each point represents the mean of three determinations. Vertical bars represent ± 1 SD. (B) Io/I vs c-phycocyanin concentration plot obtained as in Figure 2 in the absence (●) and presence (○) of DMSO (149 μg/mL). Each point represents the mean Io/I value obtained from three determinations.

Inhibition of liver microsomal lipid peroxidation induced by Fe^{+2}-ascorbic acid

Rat liver microsomes were prepared by differential ultracentrifugation as previously described [12] and stored at −80 °C until use. Protein was assayed by the Lowry method [13].

The procedure was carried out as described [14]. The microsomes (final concentration 1.3 mg protein/ml) were incubated at 37 °C in Tris buffer 50 mM pH 7.4 before induction of lipid peroxidation with 10 μM $FeSO_4$ and freshly prepared 0.2 mM ascorbic acid. The reaction was stopped by adding 0.3 mL of the incubation mixture to 2 mL of ice-cold TBA-TCA-HCl-BHT. After heating for 15 min at 80 °C and centrifugation for 15 min at 2000 g, the absorbance at 535 nm was determined. The TBA-TCA-HCl solution was prepared by dissolving 41.6 mg TBA/10 mL TCA (16.8% w/v in 0.125 N HCl). To 10 mL TBA-TCA-HCL, 1 mL of BHT (1.5 mg/mL ethanol) was added.

All the spectrophotometric measurements were done in a Spekol 220 from Carl Zeiss (Jena, Germany).

GO-induced inflammation in mouse paw

The animal model used was described by Spillert et al. [15]. Phycocyanin (50, 100 or 200 mg/Kg in saline) or DMSO (1 g/Kg), as positive control, were administered orally and i.p, respectively, to male OF1 mice. One hour later, the mice were injected in the right hind foot with 50 μL of physiological saline and in the left foot with 50 μL of 100 U/mL GO. The animals were killed at 1.5 h post injection, both hind feet amputated at the tibiotarsal joint and each paw weighed. The

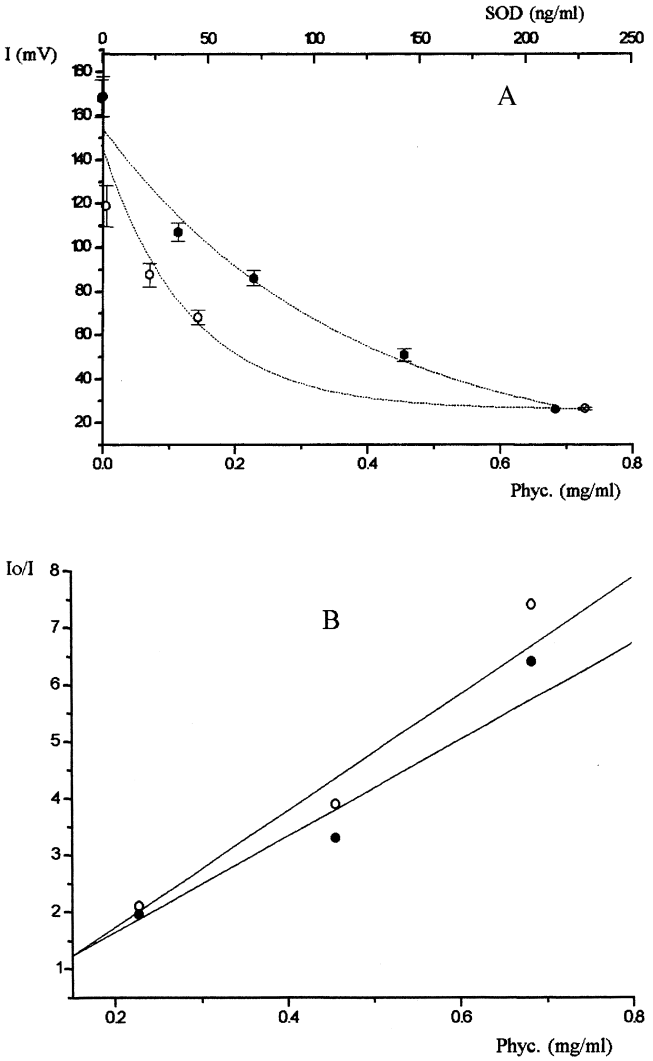

Fig. 3. Effect of various c-phycocyanin concentrations on chemiluminescence produced in the luminol-XO-HX system. (A) Decrease in chemiluminescence signal (mV) with increasing c-phycocyanin (●) or SOD (○) concentrations. Each point represents the mean of three determinations. Vertical bars represent ± 1 SD. (B) Io/I vs c-phycocyanin concentration plot obtained as in Figure 2 in the absence (●) and presence (○) of SOD (23 ng/mL). Each point represents the mean Io/I value from three determinations.

difference in weight of hind paws of each animal was called the edema index (EI).

Statistical analysis

One-way ANOVA followed by Duncan's multiple comparison test were used to calculate the significance of the differences between the means. The IC_{50} was calculated using a GraphPad InPlot software (GraphPad Software Inc., version 4.03, 1992).

Results

Chemiluminescent measurements

The CL produced by the reaction of t-BOOH with ferrous ion was used for evaluation of alkoxyl radical scavenging capacity of c-phycocyanin. The results show that c-phycocyanin inhibited the CL in this system (Fig. 1A). A comparison with trolox, a water soluble analogue of vitamin E, indicates that 0.038 μg/mL of trolox causes approximately the same effect as 76 μg/mL of c-phycocyanin in terms of 50% inhibition of the CL produced in this system.

C-phycocyanin was evaluated as a scavenger of hydroxyl radicals by determining the inhibition of CL produced by the Fenton reaction with luminol. As shown in Figure 2A, the chemiluminescence signal (mV) was inhibited by increasing phycocyanin concentrations. In this system, 0.91 mg/mL of phycocyanin caused the same inhibition (50%) as 0.125 mg/mL of DMSO, which was used as control.

In the HX-XO chemiluminescence system (Fig. 3A), phycocyanin inhibited the signal in a dose-dependent manner. However, when the HX-XO reaction was used to reduce nitroblue tetrazolium (NBT) dye, no inhibitory effect was observed (data not shown). In the double quenching experiments (Fig. 1B and 2B), the slope decreased when both phycocyanin and the specific scavenger were added together, indicating competition for the radical generated. This behaviour was not observed in the O_2^--generator system (Fig. 3B) in which the slope is independent of the previous addition of the specific scavenger (SOD) and is even increased.

Effect of phycocyanin on the CL response of PMNLs

Human leukocytes stimulated with opsonized yeast cells produced a typical time-dependent CL response with a maximal intensity at approximately 16 min followed by a decline. Table 1 shows the means of the areas under the curve (AUC) obtained for each phycocyanin concentration

Table 1. Effect of phycocyanin on the chemiluminescence response of human leukocytes.

	AUC $\bar{X} \pm SD$	Inhibition %
Control	6662 ± 221.3^a	–
Phycocyanin 1 mg/mL	5800 ± 246.5^b	12.9
2 mg/mL	4647 ± 227.0^c	30.2
3 mg/mL	3841 ± 296.5^d	42.3

Different letters: $p < 0.05$ vs. each other, $n = 5$.

Table 2. Effect of phycocyanin on GO-induced inflammation in the mouse paw.

Treatment	Edema index (g)	Inhibition %
DMSO (1 g/Kg)	0.045 ± 0.007^a	64.05
Phycocyanin 50 mg/Kg	0.110 ± 0.010^b	4.43
100 mg/Kg	0.080 ± 0.007^c	35.46
200 mg/Kg	0.061 ± 0.009^d	51.05
Glucose oxidase 100 U/mL	0.125 ± 0.008^b	–

Different letters: $p < 0.05$ vs. each other, $n = 7$ mice per group. Phycocyanin was administered orally 1 h before GO. The oedema was measured 1.5 h after injection of GO into the paw. The oedema index indicates the difference in weight between the hind paws.

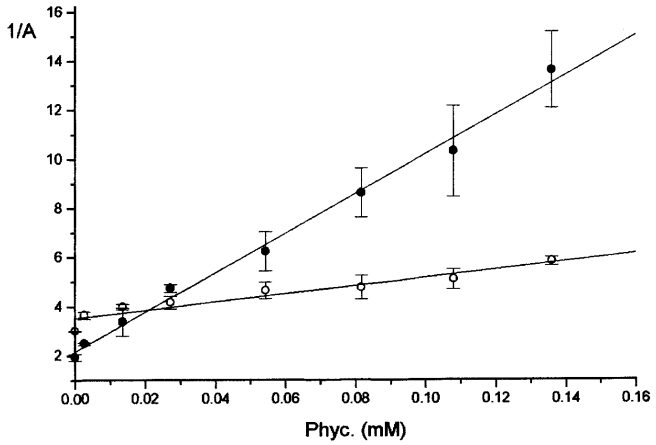

Fig. 4. Plots of $1/A_{532nm}$ against concentration of c-phycocyanin using data obtained from deoxyribose oxidation in the presence (●) or absence (○) of EDTA. Values for each point are means of three experiments. Vertical bars represent ± 1 SD.

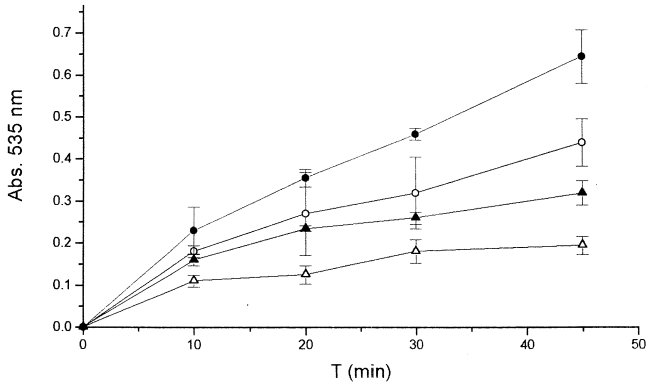

Fig. 5. Time course of microsomal non-enzymatic lipid peroxidation as affected by various concentrations of c-phycocyanin: (●) no addition, (○) 8 mg/mL, (▲) 12 mg/mL, (△) 20 mg/mL. Values for each point are means of three determinations. Vertical bars represent ± 1 SD.

and control. The CL signal was significantly reduced with respect to control by increasing phycocyanin concentrations. Significant statistical differences were also obtained among phycocyanin concentrations.

Deoxyribose assay

In the deoxyribose assay, a more conventional method, phycocyanin inhibited the deoxyribose damage in a concentration-dependent fashion. The IC_{50} calculated for c-phycocyanin was 0.86 mg/mL. In this system, a typical competition profile was obtained (Fig. 4). This was suitable for the calculation of a second-order rate constant of 3.56×10^{11} M^{-1} S^{-1}, considering a molecular weight of 36 700 g/mol for c-phycocyanin monomer [16].

Microsomal lipid peroxidation inhibition

Addition of c-phycocyanin (8, 12, 20 mg/mL) to isolated microsomes in the presence of Fe^{+2}-ascorbate, resulted in a concentration dependent decrease in lipid peroxidation ($IC_{50} = 12$ mg/ml). Both the rate and the final extent of lipid peroxidation were reduced by adding c-phycocyanin (Fig. 5).

In the presence of iron alone, c-phycocyanin did not cause lipid peroxidation, indicating that it does not have pro-oxidant effect in this system. Furthermore, its inhibitory effect was exerted during the incubation time, since it had not effect when it was added with the TBA reagent.

GO-induced inflammation in mouse paw

Differences in weight between the hind paws of animals in the various groups are shown in Table 2. Phycocyanin at doses of 100 and 200 mg/Kg orally was able to inhibit significantly peroxide-induced inflammation in a dose dependent fashion with a ED_{50} of 170.3 ± 1.62 mg/Kg. DMSO was used as a positive control and it also inhibited the inflammatory response induced by GO in mouse paw.

Discussion

In this study, we have applied established in vitro and in vivo assays in order to evaluate the antioxidant action of c-phycocyanin. This natural product was able to scavenge alkoxy and hydroxyl radicals. Two methods were used to evaluate hydroxyl radical scavenging by c-phycocyanin because hydroxyl radical is one of the most potent oxidizing species and its extreme reactivity naturally poses problems with regard to its detection [17]. In both methods, inhibition was observed at relatively high concentrations of the product.

Phycocyanin also quenched the CL signal generated in the HX-XO system, but this effect can not be ascribed to O_2^- scavenging, as demonstrated by double-quenching and NBT reduction assays. One possible explanation for this behaviour is that phycocyanin quenches CL by binding to an intermediate or co-oxidising species that may be involved in the CL reaction [18].

The inhibitory effect observed on microsomal lipid peroxidation most probably is due to a metal-binding capacity of c-phycocyanin, since chain-breaking antioxidants often introduce a lag period into the peroxidation process, corresponding to the time taken for the antioxidant to be consumed, whereas metal-binding antioxidants will give a constant inhibition throughout the reaction [19]. Another indication of such an action, is the ability of c-phycocyanin to inhibit deoxyribose damage in a site-specific manner (in the absence of EDTA) [19]. Further experiments must be performed to obtain more evidence for the ability of phycocyanin to chelate metal ions.

In the deoxyribose assay, a second-order rate constant calculated for phycocyanin was similar to that obtained, by the same method, for some non-steroidal anti-inflammatory drugs, such as indomethacin and ibuprofen (1.8×10^{10} $M^{-1}S^{-1}$) [20].

Chemiluminescence of PMNLs is the final result of luminol oxidation by strong oxidants, such as oxygen radicals and peroxides, emanating from enzymatic reactions. For addition to the myeloperoxidase-H_2O_2-halide system, the release of arachidonic acid by phospholipase A_2 and of diacylglycerol and inositol trisphosphate by phospholipase C, the metabolism of arachidonic acid by the

cyclooxygenase and lipooxygenase pathways, the activation of membrane NADPH oxidase by diacylglycerol and calcium mobilization by inositol trisphosphate are all able to induce the CL reaction. Inhibition of any of these mechanisms suppresses the CL response [21].

Phycocyanin was able to inhibit the LCL in a dose-dependent fashion, most probably through its capacity to scavenge free radicals ($OH^{\bullet}$, H_2O_2, $RO^{\bullet}$) and peroxides arising during the respiratory burst of phagocytic cells. However, it is also possible that phycocyanin could diminish CL signals in other ways, e.g. by affecting enzymes involved in the production of reactive oxygen species by activated phagocytes, NADPH oxidase and myeloperoxidase, or by interfering either with the binding of the stimulant or the arachidonic acid metabolism pathway. In this regard, we have recent evidence for inhibition by phycocyanin of LTB_4 release in an animal model of inflammation (manuscript in preparation).

The peroxide-induced inflammatory response is a valuable in vivo model in order to test agents with potential scavenging effect against H_2O_2 and $OH^{\bullet}$. GO injected into the mouse paw reacts with endogenous glucose and generates H_2O_2 which subsequently produces $OH^{\bullet}$ radicals; both together are responsible for tissue damage and for the accompanying inflammatory changes [15]. Phycocyanin reduced the edema produced by glucose oxidase in the mouse paw. This anti-inflammatory effect must be due, at least in part, to the scavenging of hydroxyl radicals, taking into account the fact that DMSO, a well known scavenger of $OH^{\bullet}$ radicals, also inhibited the inflammatory response induced by GO.

Currently, there is a consensus that much of the damage induced by H_2O_2 in vivo is due to its conversion to highly-reactive oxidants, mainly $OH^{\bullet}$ [19]. Therefore, the scavenging action of phycocyanin against OH is probably relevant to its anti-inflammatory effects.

Very recently, research carried out in our laboratory has confirmed the anti-inflammatory effects of phycocyanin in other experimental models of inflammation such as cotton pellet granuloma in the rat and TPA-induced inflammatory response in the mouse ear (manuscript in preparation).

Finally, to our knowledge, this is the first time that both antioxidant and anti-inflammatory properties have been described for c-phycocyanin.

References

[1] Duerring M, Schmidt GB, Huber R. Isolation, crystallization, crystal structure analysis and refinement of constitutive c-phycocyanin from the chromatically adapting Cyanobacterium fremyella diplosiphon at 1.66 Å resolution. J Mol Biol 1991;217:577–92.

[2] Oh Eocha C. Phycobilins. In: Lewin RA, editor. Physiology and Biochemistry of Algae. New York: Academic Press, 1962: 421–35.

[3] MacColl R, Guard-Friar D. Phycobiliproteins. Boca Raton: CRC Press, 1987:1–10.

[4] Richmond A. Large scale microalgal culture and applications. In: Round Chapman, editors. Progress in Phycological Research. Vol. 7. Biopress Ltd., 1990:8.

[5] Stocker R, Yamamoto Y, Mc Donagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043–6.

[6] Kehrer JP. Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol 1993;23:21–48.

[7] Neufeld GJ, Riggs AF. Aggregation properties of c-phycocyanin from Anacystis nidulans. Biochem Biophys Acta 1969;181: 234–43.

[8] Pascual C, González R, Romay C. Drug effects on superoxide generation and chemiluminescence response of human leukocytes. Agents Actions 1991;32:277–81.

[9] Pascual C, Romay C. Effect of antioxidants on chemiluminescence produced by reactive oxygen species. J Biolumin Chemilumin 1992;7:123–32.

[10] LKB-Wallac 1251 Luminometer Manual. The study of phagocytosis and opsonisation using luminol enhanced chemiluminiscence. Application Note 513. Wallac of Finland, 1985.

[11] Aruoma OI. Deoxyribose assay for detecting hydroxyl radicals. Meth Enzymol 1994;233:57–66.

[12] Engineer FN, Sridnar R. Inhibition of rat heart and liver microsomal lipid peroxidation by nifedipine. Biochem Pharmacol 1989;38:1279–85.

[13] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193: 265–75.

[14] Demopoulos VJ, Rekka E. Effect of aminoethylpyrroles on carragenan-induced inflammation and on lipid peroxidation in rats: Some structural aspects. J Pharm Pharmacol 1994;46:740–4.

[15] Spillert CR, Pelosi MA, Parmer LP, Lazaro EJ. A peroxide-induced inflammation model for drug testing. Agents Actions 1987;21:297–8.

[16] Oi VT, Glazer AN, Stryer L. Fluorescence phycobiliproteins conjugates for analyses of cell and molecules. J Cell Biol 1982; 93:981–96.

[17] Weber GF. The measurement of oxygen-derived free radicals and related substances in medicine. J Clin Chem Clin Biochem 1990; 28:569–603.

[18] Hodgson EK, Fridovich I. The role of superoxide in the chemiluminescence of luminol. Photochem Photobiol 1973;18: 451–5.

[19] Halliwell B. How to characterize a biological antioxidant. Free Rad Res Comm 1990;9:1–32.

[20] Parij N, Nagy AN, Neve J. Linear and non-linear competition plots in the deoxyribose assay for determination of rate constants for reaction of non steroidal anti-inflammatory drugs with hydroxyl radicals. Free Rad Res 1995;23:571–9.

[21] Lindena J, Burkhardt H, Dwenger A. Mechanisms of non-opsonized zymosan-induced and luminol-enhanced chemiluminescence in whole blood and isolated phagocytes. J Clin Chem Clin Biochem 1987;25:765–78.