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Letter to the Editor

Phycocyanin is an Antioxidant Protector of Human Erythrocytes Against Lysis by Peroxyl Radicals

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Previously we reported that phycocyanin, a biliprotein found in the blue green algae *Spirulina*, exerts scavenging action against reactive oxygen species such as $OH^{\cdot}$ and $RO^{\cdot}$ radicals and produces a concentration-dependent decrease in lipid peroxidation induced by Fe^{2+}-ascorbic acid in rat liver microsomes (Romay et al 1998a). Recently we found that bilin groups in phycocyanin, which strongly absorb at 620 nm, are readily bleached by peroxyl radicals produced in the thermolysis of 2,2′-azobis(2-amidinopropane) (AAPH) and that micromolar concentrations of phycocyanin are able to reduce the steady-state concentration of the peroxyl radicals by half, indicating a high antioxidant activity for this compound (Lissi et al 2000). Therefore, we decided to evaluate the antioxidant activity of phycocyanin by determining its protection of erythrocytes against haemolysis induced by the azo compound versus other known antioxidants including trolox (a vitamin E derivative) and ascorbic acid.

Erythrocytes from healthy donors were separated by centrifugation and washed three times with 10 volumes of saline. An assay for haemolysis mediated by peroxyl radicals was carried out according to the method of Sugiyama et al (1993). A 20% suspension of erythrocytes in 0·34 M NaCl/10 mM phosphate buffer, pH 7·4, was added to the same volume of 150 mM AAPH solution with or without phycocyanin (from *Spirulina maxima* and purified according to Neufeld & Riggs (1969)), trolox or ascorbic acid at different concentrations. The reaction mixture was shaken gently while being incubated at 37°C for 180 min. After incubation, the reaction mixture for each antioxidant concentration was diluted with appropriate volumes of buffered NaCl or distilled water, and centrifuged at 1000 *g* for 10 min. The absorbance of the supernatants was read at 540 nm.

When the water-soluble radical initiator, AAPH, was added to the suspension of erythrocytes, it induced haemolysis dose and time dependently (results not shown). Haemolysis was not observed in the absence of oxygen, suggesting the importance of peroxyl radical-mediated chain oxidation. We found that phycocyanin, at tested concentrations, inhibited erythrocyte haemolysis in the same way as trolox and ascorbic acid, well-known antioxidants (Table 1). Based on IC50 values (concentration of the additive that gave 50% inhibition of peroxidative damage), phycocyanin proved to be almost sixteen times more efficient as an antioxidant than trolox and about twenty times more efficient than ascorbic acid.

It has been shown previously that free radicals generated from AAPH induce chain oxidation of lipids and proteins in erythrocyte membranes and, eventually, cause haemolysis, which is directly proportional to the total flux of peroxyl radicals (Miki et al 1987). It has also been shown that water-soluble radical scavengers such as uric acid, ascorbic acid and trolox scavenge the peroxyl radicals derived from AAPH in the aqueous phase efficiently before the radicals can attack the erythrocyte membrane, thus protecting it from oxidative damage (Niki et al 1988).

It is now accepted that free radicals, especially active oxygen-centered radicals such as hydroxyl, alkoxyl and peroxyl radicals, attack lipids, carbohydrates, proteins and DNA to induce membrane damage, protein modification, enzyme inactivation and strand break and base modification of DNA, eventually leading to a variety of pathological events such as cancer and aging (Sies 1991). Red blood cell membranes are prone to lipid peroxidation because of their high polyunsaturated lipid content and because they are directly exposed to molecular oxygen and haemoglobin.

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Table 1. Percentage of haemolysis induced by peroxyl radicals in human erythrocytes and its inhibition by antioxidants.

Additive	Haemolysis (%)	Protection by additive (%)	IC50(mM)
None	92·2 ± 5·4	–	–
Phycocyanin			
0·012 mM	88·0 ± 2·4	12·0	
0·025 mM	75·5 ± 8·2	24·5	0·035
0·05 mM	12·5 ± 1·0	87·5	
0·075 mM	2·3 ± 0·5	97·7	
Trolox			
0·5 mM	78·5 ± 3·3	21·5	
0·6 mM	40·3 ± 7·5	59·7	0·57
0·7 mM	21·8 ± 7·0	78·2	
Ascorbic acid			
0·5 mM	86·6 ± 3·5	13·4	
0·8 mM	15·7 ± 1·5	84·3	0·71
1·0 mM	10·3 ± 0·5	89·7	

Percentage of haemolysis was calculated by the relation A/B × 100%, where A and B are the absorbance of the erythrocyte dilution in NaCl and distilled waters respectively. The experiments were repeated 4 times at each concentration of additive used.

Phycocyanin exerts anti-inflammatory activity in various in-vitro and in-vivo experimental models (Romay et al 1998a, b; González et al 1999) and has a hepatoprotective effect against carbon tetrachloride- and *R*-(+)-pulegone-mediated toxicity in rats (Vadiraja et al 1998).

Our results provide evidence of the protective effect of phycocyanin against haemolysis induced by peroxyl radicals in human erythrocytes, which seems to be due to the scavenging action of the radicals in the aqueous phase. Thus inhibiting chain of initiation of lipid peroxidation, in a similar manner to trolox and ascorbic acid.

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