ANTIOXIDANT ACTIVITY OF SOME B COMPLEX VITAMINES; A PRELIMINARY STUDY

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Summary

The antioxidant activities of B complex vitamins were evaluate with employing 3 different in vitro systems such as DPPH, H_2O_2 radicals scavenging and reducing power. They also showed the significant suppression against Hydrogen peroxide scavenging. IC_{50} for H_2O_2 radical-scavenging activity was in the order: Riboflavin> Thiamine> Cyanocobalamin> Folic Acid> Niacin> Pantothenic acid> Pyridoxine> Biotin, respectively. Thiamine exhibited potent reducing power activity that was comparable with vitamin C. All of the vitamins exhibited good activity in DPPH and H_2O_2 radical scavenging. These results suggest a possibility that hydrogen peroxide scavenging may be a mechanism for antioxidant activity of some B complex vitamins.

Key words: B complex vitamins, radical-scavenging, DPPH

Introduction

Free radicals ,defined as molecules having an unpaired electron in the outer orbit are continuously, being formed in aerobic metabolisms (1, 2) .however Many of them may play an important role in the origin of life and biological evolution , But they can damage biologically important macromolecules including DNA, proteins and membrane lipids that being implicated in the etiology of several diseases such as cancer and neurodegenerative diseases. Organisms to control excess production of free radicals evolved a many of enzymatic and nonenzymatic endogenous antioxidant defenses mechanisms (3, 4). Hence Endogenous antioxidant defense is insufficient to prevent oxidative damage, dietary antioxidants may be particularly important in diminishing the cumulative effects of oxidative damaged molecules. Some vitamins inhibit NO production by inhibiting NO synthase, in support of their known antiatherogenic and antineuroinflammatory roles. And the some Vitamins directly scavenge ROS and up regulate the activities of antioxidant enzymes.

Among them, vitamin E has been recognized as one of the most important antioxidants (5). On the other hand, recently antioxidant and radical-scavenging activities of the aqueous extract of rice bran and the active principles for these activities were reported (6). Since aqueous extract of rice bran is rich in B complex vitamins, we investigated the antioxidant and radical-scavenging activities of B complex vitamins by some *in vitro* experiments. Among the B complex radical-scavenging activities of thiamin and its derivatives previously reported by hydroxyl radical-scavenging, hydro peroxide generation from linoleic acid peroxidation and oxygen radical generation in human blood neutrophils methods (7). In the present study we examined the antioxidant activities of some B complex vitamins with 3 in vitro assay systems including: DPPH, H_20_2 radicals scavenging and reducing power.

Materials and methods

Chemicals: Trichloroacetic acid (TCA), 1,1-diphenyl-2-picryl hydrazyl (DPPH), potassium ferricyanide and Hydrogen peroxide (H_2O_2) were purchased from Sigma Chemicals Co. (USA). Butylated hydroxyanisole (BHA), ascorbic acid and ferric chloride were purchased from Merck (Germany). Vitamins Purchased from Merck Company.

DPPH Radical-Scavenging Activity: The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the samples (8). Different concentrations of each sample were added, at an equal volume, to methanolic solution of DPPH (100 μM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C, BHA and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Reducing Power Determination: The reducing powers of B complex Vitamins were determined according to method of our recent published articles (8, 9). 2.5 ml of each samples (25-800 μg ml^{-1}) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K_3 $Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Scavenging of Hydrogen Peroxide: A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). samples (0.1-1 mg ml^{-1}) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the samples and standard compounds was calculated as follows: % Scavenged $[H_2O_2] = [(A_o - A_1)/A_o] \times 100$ where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the samples and standard (11).

Statistical Analysis: Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range test. The EC_{50} values were calculated from linear regression analysis.

Results and discussion

DPPH Radical-Scavenging Activity: The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (12, 13). It was found that the radical-scavenging activities of all the samples increased with increasing concentration. IC_{50} for DPPH radical-scavenging activity exist in Table 1. Riboflavin showed the highest activity (IC_{50} = 480.5 ± 21 μg ml^{-1}).

Hydrogen Peroxide Scavenging: Scavenging of H_2O_2 by samples may be attributed to their phenols, which can donate electrons to H_2O_2, thus neutralizing it to water. The samples were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Results exist in Table 1. Folic Acid showed good activity that was comparable with quercetin ($p > 0.05$). Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems (14).

Sample	DPPH free radical scavenging, IC_{50} (μg ml^{-1})	H_2O_2 scavenging activity, IC_{50} (μg ml^{-1})
Thiamine	523.4 ± 23	116.6 ± 6.4
Riboflavin	480.5 ± 21	335 ± 17
Niacin	716.4 ± 34	368 ± 24
Pantothenic acid	779.22 ± 30	176 ± 11
Pyridoxine	811.8 ± 26	158 ± 9.2
Biotin	904.14 ± 38	500.3 ± 18
Folic Acid	711.18 ± 31	47.08 ± 1.6
Cyanocobalamin	624.42 ± 27	284.6 ± 11
BHA	53.96 ± 2.4	-
Vit C	5.05 ± 1.8	21.4 ± 1.3
Quercetin	5.28 ± 2.2	52 ± 1.9

Table 1. DPPH and H_20_2 radicals scavenging activity of B complex vitamins

Reducing Power: In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm (15,16) Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 1 shows the dose response curves for the reducing powers of the samples. It was found that the reducing powers of all the samples also increased with the increase of their concentrations. Thiamin exhibited potent reducing power that was comparable with Vitamin C ($p > 0.05$). Thiamin molecular structure appear to function as good electron and hydrogen atom donors and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products.

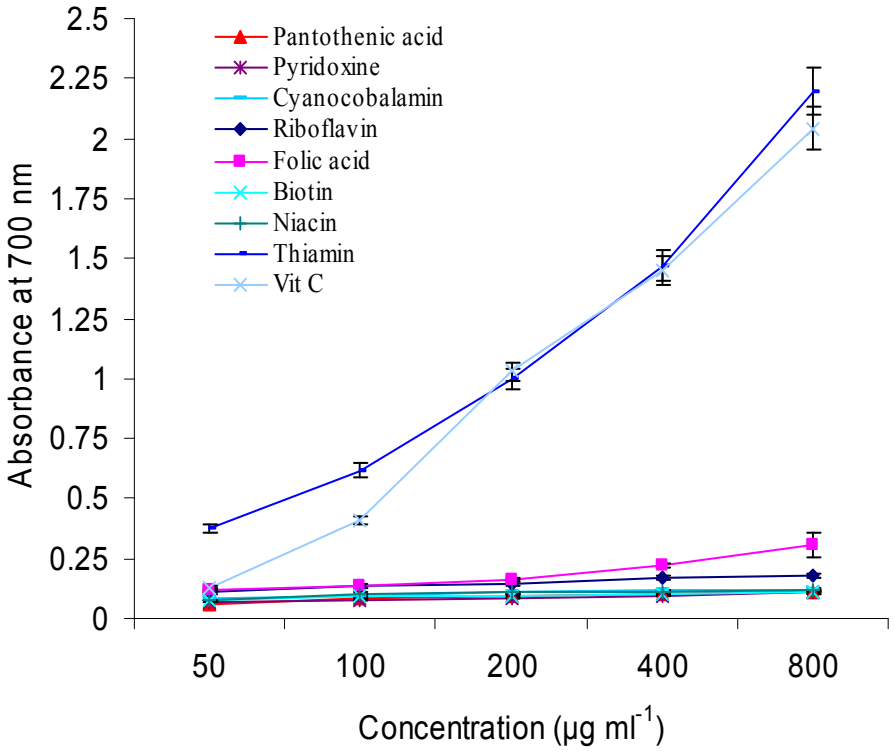

Fig.1. Reducing powers of B complex vitamins. Vitamin C used as control.

Previously, B complex vitamins were considered to be non-antioxidant vitamins. However recent studies showed that vitamin B6 family such as pyridoxine and pyridoxamine caused suppressive effect on glucose- induced lipid peroxidation and super oxide generation in diabetic models experiments (17). These results indicate a possibility that some of B complex vitamins possess potent antioxidant activity. Further in vivo antioxidant activities and in different antioxidant mechanisms is needed.

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