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Maltol, an Antioxidant Component of Korean Red Ginseng, Shows Little Prooxidant Activity

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Some antioxidant phenolic compounds exhibit prooxidant activity mainly due to their abilities to reduce Fe^{3+} to Fe^{2+}. Reducing ability and prooxidant activity of maltol, an antioxidant component of Korean red ginseng, were compared with those of pyrogallol. Maltol at 2 mM did not appreciably reduce Fe^{3+} to Fe^{2+} and also failed to reduce nitroblue tetrazolium. Stimulation of hydroxyl radical mediated-deoxyribose degradation by pyrogallol was maximal at 60 μM. Maltol stimulated the deoxyribose degradation to a much less extent, and a similar stimulatory effect was observed at a concentration of more than 100-fold higher than that of pyrogallol. The stimulatory effect of maltol reached a plateau over 1 mM, suggesting the removal of hydroxyl radicals by excess maltol. In bleomycin-Fe^{3+}-DNA assay, maltol at 2 mM produced a 2.5-fold increase of the iron-bleomycin-dependent DNA degradation over the basal value, whereas pyrogallol at 10 μM accelerated DNA degradation by ca. 10-fold. Furthermore, maltol inhibited Fe^{2+}-stimulated DNA degradation by bleomycin. These results strongly suggested that maltol is an antioxidant with little prooxidant activity.

Key words : Maltol, Antioxidant, Prooxidant activity, Deoxyribose method, Bleomycin assay.

INTRODUCTION

Oxidative stress has been associated with various pathological conditions such as inflammation, cancer, artherosclerosis etc., and antioxidant components from plants have been extensively studied for the prophylaxis and/or treatment of these disease states. For example, an antioxidant is potentially antiatherogenic by inhibiting the oxidation of LDL or by protecting the endothelium from oxidative damage (reviewed in Jackson *et al.*, 1993; Brune *et al.*, 1991). We have previously isolated maltol (3-hydroxy-2-methyl-4-pyrone) as one of the antioxidant principles from *Panax ginseng* C.A. Meyer (Han *et al.*, 1985).

Some antioxidants such as plant phenolics, however, have often been demonstrated to exhibit prooxidant activity as well as antioxidant activity (Laughton *et al.*, 1989). In addition to iron-chelating ability, some phenolic compounds are able to reduce Fe^{3+} to Fe^{2+} (Boyer *et al.*, 1988). Fe^{2+} ions participate in free radical reactions and these iron-catalyzed free radical damage has been indicated to play an important role in numerous diseases and side effects of drugs (Halliwell, 1995; Halliwell and Gutteridge, 1986). On the other hand, maltol forms a stable complex with Fe^{3+} ions and the Fe^{3+}-maltol complex was proposed to be used as an iron delivery agent in the treatment of iron deficiency (Barrand and Callingham, 1991).

In this study, we provide evidence that maltol shows little prooxidant activity by studying the reducing ability of maltol and its effect on iron-dependent free radical generating systems.

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MATERIALS AND METHODS

L-Ascorbic acid, bathophenanthrolinedisulfonic acid (BPS), deoxyribose, DNA (type I from calf thymus), nitroblue tetrazolium (NBT), and 2-thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Maltol was from Aldrich (Milwaukee, WI, USA); bleomycin (BLEOCINA) from Dong-A Pharm. Co. (Seoul, Korea); pyrogallol from Yakuri Pure Chemicals (Osaka, Japan).

Reducing abilities of maltol and pyrogallol

(1) Reduction of NBT : According to the literature procedure (Gutteridge and Fu, 1981), 0.1 ml of EDTA (final concentration of 0.1 mM), 0.1 ml of NBT (1 mM) and 0.1 ml of test compounds in 0.7 ml of 10

mM phosphate-buffered saline (PBS, pH 7.4) were incubated at 37°C for 20 min. Following addition of 2.0 ml of PBS, ΔA_{540} values were measured.

(2) Reduction of Fe^{3+} : $FeCl_3$ (0.1 ml, 0.05 mM), BPS (0.1 ml, 0.1 mg/ml) and 0.1 ml of test compounds at varying concentrations in 50 mM PBS were reacted together at 25°C for 10 min in 0.7 ml of 50 mM PBS (pH 7.4) before the addition of 0.1 ml 6% (v/v) orthophosphoric acid, and the absorbance at 532 nm was measured.

Effects of maltol on hydroxyl radical (• OH) generation by Fe^{3+}-EDTA and H_2O_2

According to the method of Laughton *et al.* (1989), hydroxyl radicals were generated by incubating the following reagents in a volume of 1.2 ml for 40 min at 37°C : 10 mM PBS, pH 7.4, H_2O_2 (1.42 mM), $FeCl_3$ (20 μM), EDTA (100 μM), deoxyribose (2.8 mM) and ascorbate or test compounds. The extent of deoxyribose degradation by • OH (prooxidant activity of a sample) was determined with ΔA_{532} that measures the TBA-reactive products formed by the reaction of · OH with deoxyribose.

Measurement of prooxidant activity using DNA-bleomycin assay

Triplicate 1.0 ml samples of DNA (0.5 mg/ml), bleomycin (0.045 mg/ml), $MgCl_2$ (5 mM), $FeCl_3$ (50 μM), test compounds at varying concentrations in 50 mM Tris/HCl buffer, pH 7.4, were incubated at 37°C for 20 min, and DNA degradation products were measured by the TBA method (Gutteridge *et al.*, 1981).

DNA-bleomycin assay with Fe^{2+} and maltol

Triplicate 1.0 ml samples containing DNA (0.5 mg/ml), bleomycin (0.045 mg/ml), $MgCl_2$ (5 mM), $FeSO_4$ (50 μM), and maltol at varying concentrations in 50 mM Tris/HCl buffer, pH 7.4 were incubated at 37°C for 40 min, and DNA degradation products were measured by the TBA method. $FeSO_4$ was omitted in the blank, and maltol was not added to the control sample.

RESULTS AND DISCUSSION

A marked difference in reducing ability of maltol and pyrogallol was observed (Table I). Maltol at 10 times higher concentration showed only 20% reducing ability of pyrogallol. Maltol also failed to reduce NBT even at 2 fold molar excess. Some plant flavonoids have been reported to be able to reduce Fe^{3+} bound to ferritin to Fe^{2+}, thus releasing "free" iron ions which in turn may accelerate oxidative damage under certain circumstances (Boyer *et al.*, 1988). Data obtained in this study indicated that maltol is expected not to deprive iron ions from iron transport proteins.

Table I. Reducing ability of maltol and pyrogallol

	Reduction of NBT (ΔA_{540})	Reduction of Fe^{3+} (ΔA_{532})
Maltol (mM)		
0.1	0.0	0.0
0.4	0.0	0.025
1.0	0.0	0.043
2.0	n.d.	0.051
Pyrogallol (mM)		
0.01	0.0	0.042
0.06	0.0	0.087
0.1	0.070	0.18
0.2	0.12	0.26
0.5	0.21	0.38

Results are the means of three determinations. Reduction of 0.1 mM NBT and 50 μM Fe^{3+} ions by maltol and pyrogallol at indicated concentrations was measured by ΔA_{540} and ΔA_{532}, respectively. n.d.: not determined.

Table II. Effects of maltol and pyrogallol on hydroxyl radical generation in the presence of Fe^{3+}-EDTA

Additions		ΔA_{532}
Blank (without deoxyribose)		0.00
Control (without Fe^{3+}-EDTA)		0.16
Control (without ascorbate)		0.16
Ascorbate (1 mM)		0.32
Ascorbate (1 mM) + DMSO (20 mM)		0.18
Maltol (mM)	0.1	0.18
	0.4	0.28
	0.8	0.29
	1.2	0.27
	2.0	0.27
Pyrogallol (mM)	0.01	0.32
	0.02	0.39
	0.04	0.47
	0.06	0.72
	0.08	0.64

Results are the means of three determinations. All tubes (1.2 ml) contained H_2O_2, $FeCl_3$-EDTA, deoxyribose, ascorbate and test compound as indicated. Increase in absorbance at 532 nm was measured against the blank in which deoxyribose was omitted, as described in MATERIALS AND METHODS.

Effect of maltol on iron-catalyzed, hydroxyl radical-mediate degradation of deoxyribose was compared to those of pyrogallol and a biological antioxidant, ascorbate. As shown in Table II, ascorbate at 1 mM reduced Fe^{3+}-EDTA to Fe^{2+}-EDTA, which reacted with H_2O_2 to produce hydroxyl radicals. A known radical scavenger, DMSO inhibited deoxyribose degradation confirming that hydroxyl radicals were involved. In this system, pyrogallol containing three adjacent hydroxyl groups acted as a powerful reducing agent. Stimulation of hydroxyl radical mediate-deoxyribose degradation by pyrogallol was maximal at around 60 μM, and decreased at higher concentrations (see be-

Table III. Effect of maltol and pyrogallol on DNA degradation in a bleomycin-Fe^{3+}-DNA system

Additions	ΔA_{532}
Blank (without Fe^{3+})	0.0
Control	0.026
Maltol (mM)	
0.4	0.041
0.8	0.048
1.6	0.058
2.0	0.064
Pyrogallol (mM)	
0.01	0.24
0.05	0.83
0.10	0.54
0.50	0.34
1.0	0.23

Results are the means of three determinations. All tubes (1.0 ml) contained DNA, bleomycin, $MgCl_2$, $FeCl_3$, buffer and test compound as indicated. The extent of DNA degradation was measured by the TBA method as described in MATERIALS AND METHODS.

Table IV. Concentration-dependent inhibition by maltol of Fe^{2+}-stimulated DNA degradation by bleomycin

Additions	ΔA_{532}
Blank (without Fe^{2+})	0.021
Control	0.57
Maltol (mM)	
0.5	0.45
1.0	0.38
1.5	0.29
2.0	0.26
3.0	0.24

Results are the means of three determinations. All tubes (1.0 ml) contained DNA, bleomycin, $MgCl_2$, $FeSO_4$, buffer and test compound as indicated. The extent of DNA degradation was measured by the TBA method as described in MATERIALS AND METHODS.

low). On the other hand, maltol stimulated the deoxyribose degradation to a much less extent, and a similar stimulatory effect was observed at a concentration of around 100 times lower than that of pyrogallol. The stimulatory effect of maltol reached a plateau over 1 mM. This may well be due to the removal of hydroxyl radicals by excess maltol. Protective effect by maltol against tissue damages induced by oxygen radicals has been suggested in the literature (Shin *et al.*, 1990).

The effects of maltol and pyrogallol on DNA damage by a bleomycin-Fe^{3+}-DNA complex were further investigated (Table III). The antitumor bleomycin binds Fe^{3+} ions and to DNA. In the presence of a reducing agent the ternary complex produces DNA degradation (Gutteridge and Halliwell, 1987). Maltol at 2 mM produced a 2.5 fold increase over the basal value, whereas pyrogallol at 10 μM accelerated DNA degradation by ca. 10 fold. The stimulatory effect of pyrogallol increased with concentration up to a maximum of 50 μM, but then became smaller. At higher concentrations, iron-binding capacity allows pyrogallol to withdraw iron from the bleomycin-Fe^{3+}-DNA complex (Laughton *et al.*, 1991).

Maltol inhibited Fe^{2+}-stimulated DNA damage by bleomycin (Table IV). Maltol produced a 33% inhibition at 1 mM. Bleomycin has been shown to act as a "ferroxidase" catalyzing the oxidation of Fe^{2+} to Fe^{3+}. Maltol binds to Fe^{2+} poorly; the ratio of Fe^{3+} to Fe^{2+} bound to maltol is 10^{12} (Barrand *et al.*, 1990). Rather, maltol forms a chelate with Fe^{3+} in a molar ratio of 2 : 1 or 3 : 1 depending on pH and concentration (Han *et al.*, 1985; Hider *et al.*, 1981). The high affinity of maltol for Fe^{3+} ions ($\log\beta_3$=28) may interfere with recycling of iron to Fe^{2+}.

As indicated by the Fenton reaction and iron-catalyzed Haber-Weiss reaction, the capability of reducing Fe^{3+} to Fe^{2+} is a prerequisite for a compound to have prooxidant activity. The lack of prooxidant activity of maltol demonstrated in the present study using the deoxyribose method and DNA-bleomycin assay may be explained by its low Fe^{3+}-reducing ability.

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