Maltol as an Antioxidant: Inhibition of Lipid Peroxidation and Protection of NADP-Isocitrate Dehydrogenase from the Iron-mediated Inactivation

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ABSTRACT
Maltol (3-hydroxy-2-methyl-4-pyrone) inhibited iron-mediated lipid peroxidation, determined as the formation of thiobarbituric acid-reactive substances, but dimethylpyrone, an analogue of maltol showed no effect on the formation of lipid peroxides. NADP-isocitrate dehydrogenase, a principal enzyme generating reduced NADP, was protected by maltol but not by dimethylpyrone from the ferrous ion-mediated inactivation. Protection of NADP-isocitrate dehydrogenase can enhance the supply of NADPH required for the regeneration of reduced glutathione for scavenging reactive oxygen species. Antioxidant properties of maltol were closely related to the enhanced oxidation of ferrous ion as a prooxidant, and can be explained by the electron-deficient nature of 3-hydroxypyrone ring.

Maltol (3-hydroxy-2-methyl-4-pyrone) is found in coffee, chicory, roasted malt and caramelized foods (1, 3), and is widely used as a flavoring agent to give “freshly baked flavor” to bread and cakes (1). Some Chinese herbal medicines also contain maltol as an active component (5). Biological function of maltol remains obscure, but maltol and hydroxy pyrone compounds show a potent metal-chelating activity (7). Analogues of maltol, the hydroxypyridone compounds, can prevent postischemic cardiac injury, and this protective effect is assumed to be related to the iron-chelating activity (11). Iron and copper the transition metals participate in generating reactive oxygen species through the interaction with oxygen molecule, and thus, iron chelation is responsible for scavenging reactive oxygen species (4). In this paper we report that maltol acts as an antioxidant, and analyzed its mechanism in relation to iron coordination properties of this compound. Maltol inhibits the lipid peroxidation and protects NADP-isocitrate dehydrogenase from iron-mediated inactivation by stimulating the oxidation of ferrous ion the prooxidant.

MATERIALS AND METHODS

Chemicals. Maltol, bathophenanthroline disulfonate, threeo-Ds-isocitrate and NADP are products of Sigma-Aldrich-Japan (Tokyo, Japan). Dimethylpyrone was obtained from Tokyo Fine Chemicals Co. (Tokyo, Japan). NADP-isocitrate dehydrogenase is a product of Oriental Yeast Co. (Osaka, Japan). Other chemicals were obtained from commercial sources.

Lipid peroxidation. Lipid peroxidation was determined as the formation of thiobarbituric acid-reactive substances (2) by iron/ascorbate system with liver microsomes (12).

Inactivation of NADP-isocitrate dehydrogenase. Purified NADP-isocitrate dehydrogenase of 1.5 μg was inactivated by preincubating for 2.5 min with the mixture containing 10 μM FeCl3, 0.25 mM threeo-Ds-isocitrate the substrate and 2.5 mM MgCl2 in 40 mM Tris-HCl buffer (pH 7.1) in
the absence and presence of various concentrations of pyrone compounds. Enzyme reaction was started by addition of 0.25 mM NADP to the mixture, and changes in absorbance at 340 nm were followed.

**Autooxidation of Fe^{2+} ion.** Interaction of pyrone compounds with iron was evaluated by the effect of these compounds on the rate of autooxidation of Fe^{2+} (12). The sample of 2 mL contained 10 mM Tris-HCl (pH 7.1), 0.1 mM FeSO_{4} and 0.05 mM pyrone compounds. The reaction was started by the addition of FeSO_{4}. Aliquots of 0.2 mL were mixed with 0.1 mL of 1 mM bathophenanthroline disulfonate, and absorbance at 540 nm was measured.

**RESULTS**

We examined the effect of maltol and dimethylpyrone on the lipid peroxidation of microsomes from rat liver. Incubation of microsomes with iron and ascorbate produced thiobarbituric acid-reactive substances indicative of lipid peroxidation. Antioxidant effect was determined as the inhibition of the formation of the thiobarbituric acid-reactive substances by pyrone compounds. Concentrations required for 50% inhibition of the peroxidation were about 130 and 30 μM for maltol and trolox, respectively, but dimethylpyrone did not show any inhibitory effect (Fig. 1).

NADP-isocitrate dehydrogenase, the principal NADPH generating enzyme, was irreversibly inactivated by Fe^{2+} ion, and the concentration of ferrous ion required for the enzyme inactivation was about 3 to 4 μM (Fig. 2). Fe^{2+} ion did not inactivate the enzyme (data not shown). Maltol effectively protected the enzyme from the ferrous ion-dependent inactivation (Fig. 2). Fig. 3 shows the effect of the increasing concentrations of maltol on the enzyme inactivation: the maltol concentration required for 50% protection was 130 to 140 μM, which was comparable to that required for the inhibition of lipid peroxidation. Dimethylpyrone did not show any protective effect on the iron-dependent inactivation of the enzyme (Figs. 2 and 3).

The effect of pyrone compounds on the rate of Fe^{2+} autooxidation was examined. Maltol increased the rate of Fe^{2+} oxidation effectively, but dimethylpyrone did not show any stimulating effect on the Fe^{2+} oxidation (Fig. 4).

**Fig. 1.** Effect of maltol and trolox on the iron-induced lipid peroxidation of rat liver microsomes. Lipid peroxidation was induced by 10 μM FeCl_{3}, 0.5 mM ascorbic acid and 0.2 mg microsomal fraction (Yoshino and Murakami, 1998) in the presence and absence of pyrone compounds. The mixture was incubated at 37°C for 20 min, and the reaction was stopped by addition of 100% trochloroacetic acid. Lipid peroxides were determined as the thiobarbituric acid-reactive substances (2). ●, maltol; □, dimethylpyrone; △, trolox.

**Fig. 2.** Inactivation of NADP-isocitrate dehydrogenase by Fe^{2+} ion in the presence of pyrone compound. NADP-isocitrate dehydrogenase was incubated for 2.5 min with the mixture containing 10 μM FeCl_{3}, 0.25 mM three-Ds-isocitrate, 2.5 mM MgCl_{2} and 40 mM Tris-HCl buffer (pH 7.1) in the absence and presence of pyrone compounds. Activity was determined by addition of 0.25 mM NADP. ●, no addition; □, 0.1 mM maltol added; ■, 0.2 mM maltol added; △, 0.2 mM dimethylpyrone added.
Fig. 3. Effect of increasing concentrations of pyrone compounds on the inactivation of NADP-isocitrate dehydrogenase by Fe\(^{2+}\) ion. The enzyme was inactivated by 10 \(\mu\)M FeCl\(_3\) in the presence of various concentrations of pyrone compounds as described in the legend to Fig. 2. ■, 0.2 mM maltol; ○, dimethylpyrone.

DISCUSSION

The present paper showed the antioxidant properties of maltol: maltol inhibited iron-mediated lipid peroxidation, and protected NADP-isocitrate dehydrogenase from the iron-dependent inactivation. Fe\(^{2+}\) ion-isocitrate complex binds to the metal-binding sites of NADP-isocitrate dehydrogenase and causes specific oxidative cleavage and inactivation of the enzyme (10). NADP-isocitrate dehydrogenase is a principal reaction producing NADPH for the regeneration of reduced glutathione. Reduced glutathione is utilized as the substrate of glutathione peroxidase that catalyzes the reaction scavenging hydrogen peroxide to H\(_2\)O, and protects tissues from the injury by hydroxyl radical, the most toxic reactive oxygen species. Inactivation of NADP-isocitrate dehydrogenase may disrupt the antioxidant system by decreasing the regeneration of reduced glutathione; on the contrary, the compounds protecting this enzyme can act as an antioxidant.

Antioxidant properties of maltol can be explained by the enhanced oxidation of Fe\(^{2+}\) ion, which is closely related to the electron-deficient nature of pyrone ring with unshared pair of electrons on the oxygen atom. However, no effect of dimethylpyrone on the Fe\(^{2+}\) oxidation suggests that 3-hydroxy group is necessary for the enhanced oxidation of Fe\(^{2+}\) ion. 3-Hydroxy group of maltol participates in the formation of chelation complex with iron as demonstrated in pyridone compounds (7). Pyrone ring is a rather potent base, and maltol is a resonance hybrid between carbonium and oxonium (pyrilyium) forms (Fig. 5). Maltol with 3-hydroxy group can bind iron and attracts electron from Fe\(^{2+}\) to form oxidized Fe\(^{3+}\) iron, resulting in scavenging the prooxidant Fe\(^{2+}\) ion. Previously we demonstrated that dipicolinic acid, the pyridine compound, acts as an antioxidant by enhancing oxidation of Fe\(^{2+}\) ion, the prooxidant (8, 9). Chemical properties of pyridone ring and pyrone compounds are similar to each other. Both are characterized by their electron-deficient nature and act as electron-attracting compounds, resulting in enhancing

Fig. 4. Effect of pyrone compounds on the autooxidation rate of Fe\(^{2+}\) ion. Iron oxidation was followed by determining the Fe\(^{3+}\) concentration with baphophenanthroline disulfonate (12). △, No addition; ■, 0.05 mM maltol added; □, 0.05 mM dimethylpyrone added.

Fig. 5. Resonance hybrid of maltol.

**Carbonium form**

**Oxonium form**

(Pyrilyium cation)
Fe$^{2+}$ oxidation.

Maltol is found in roasted malt and some aroma compounds isolated from soybeans and mung beans (6), and used for giving the flavor to bread (1). Habit eating food made from beans may cause an increased intake of large amount of maltol. Maltol can protect cells from oxidative damage directly as an antioxidant and further by providing reduced glutathione through the protection of NADPH-generating isocitrate dehydrogenase.

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REFERENCES