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Effects of three biological thiols on antimutagenic and antioxidant enzyme activities

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ABSTRACT

The antimutagenic and antioxidant enzyme activities of captopril (CAP), cysteine (CYS), and glutathione (GSH) were evaluated for finding concentration-dependent inhibitory effects against: (1) the mutagenicity of 2-amino-3-methylimidazo [4,5-*f*] quinoline (IQ), an indirect mutagen; and (2) *N*-methyl-*N*′-nitrosoguanidine (MNNG), a direct mutagen toward *Salmonella typhimurium* TA98 and TA100. Of the three thiols, CYS and GSH exhibited better inhibitory effects against IQ-induced mutation toward TA98 and TA100, respectively. GSH also showed a protective effect against MNNG-induced mutation toward TA98 and TA100, meanwhile, CAP showed the least inhibitory effect. CYS, GSH, and CAP also dose-dependently increased the activities of glutathione transferase, glutathione peroxidase, and glutathione reductase in hepatic BNL cells. CAP showed the superior inducing effects on glutathione transferase activity. These data suggested that the bioactive properties of biological thiols might contribute to their effects of antimutagenic activities as well as regulation on activities of antioxidant enzymes.

1. Introduction

The accumulation of DNA damage may promote the development of most cancers and degenerative diseases. The reduction of DNA damage plays an important role in aging diseases prevention. (Ferguson, Philpott, & Karunasinghe, 2004). Different reports have indicated that various environmental mutagens present in diet exhibit different mechanisms to damage DNA and induce mutation production. For example, 2-amino-3-methylimidazo [4,5-*f*] quinoline (IQ), a known carcinogenic heterocyclic aromatic amine for carcinogenicity in the liver is formed during the cooking of meat and fish (Felton & Knize, 1991). In addition, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), another strong environmental mutagen, is generally thought to induce direct methylation of DNA by methyldiazohydroxide, which is an alkylating hydrolysis product of MNNG (Niknahad & O'Brien, 1995). These damages of DNA, if not prevented, may result in DNA mutation that eventually increase the risk of tumors progression (Puisieux, Lim, Groopman, & Ozturk, 1991). Except to form direct adducts with DNA, these toxic mutagens may be metabolized and induce harmful free radicals production in cells, which also destroy the biological molecules (e.g. lipids and DNA) and cause mutations. However, these destructive free radicals may be counteracted by intercellular antioxidant enzyme systems such as glutathione peroxidase (GPX), glutathione reductase (GR), glutathione transferase (GST) and by non-enzymatic antioxidant systems in cells. In fact, due to the protective properties of antioxidation and detoxication, the antioxidant enzymes decreasing toxicities of free radicals and mutagen metabolites will play protective roles in the progression of antimutation and anticancer in organs (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006).

According to previous epidemiological studies, diets containing a large number of vegetables and fruits can help consumers against certain cancers and other relevant aging diseases. That phytochemicals present in vegetables and fruits contributing to these protective actions have been demonstrated (Boyer & Liu, 2004). A part of these phytochemicals may serve as antimutagens to reduce the production of cell mutation and cancer progression; others can decrease free radicals production to protect DNA against oxidation damage. To evaluate the potential of antimutagenesis, a number of phytochemicals have been tested as antimutagens in the Ames bacterial assays. For example, the antimutagenic activity of black tea in Ames *Salmonella* assays was attributed to both active tea polyphenols theaflavins and thearubigins (Gupta, Chaudhuri, Seth, Ganguly, & Giri, 2002). Further, catechins present in green tea might protect against reactive carcinogenesis intermediates such as free radicals and electrophiles formation during the metabolic activation of carcinogens and mutagens (Hernaez, Xu, & Dashwood, 1998). These data support the observation that the cancer preventive abilities of phytochemicals are due at least in part to their antimutagenesis and free radicals reduction activities. However, these active phytochemicals, in addition to inhibiting mutation production, may have physiological protective mechanisms against oxidative stress production in cells that are favourable for consumer health. Consequently, there have been numerous

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investigations on the bifunctional properties of naturally occurring phytochemicals.

Dietary thiols such as captopril (CAP), glutathione (GSH), and cysteine (CYS) with a sulphydryl functional group were identified in vegetables and fruits (Demirkol, Adams, & Ercal, 2004). More dietary thiols supplements could regulate cell and tissue thiol levels in different models (Arakawa et al., 2006). Cellular thiols redox status can modulate physiological metabolisms, including xenobiotic detoxification and antioxidant system operation (Fang, Yang, & Wu, 2002). These studies imply that cellular activities of antioxidative enzymes might be regulated by dietary thiols. Among these dietary thiols, CAP, an inhibitor of angiotensin-converting enzyme, has exhibited chemoprotective effects against cyclophosphamide-induced genotoxicity in bone marrow cells (Hosseinimehr & Karami, 2005). Similarly, GSH, a known antioxidant, can conjugate with toxic metabolites produced in the process of mutagens activation and detoxification (Peklak-Scott, Townsend, & Morrow, 2005). CYS, a precursor of metallothioneins and GSH biosynthesis, is particularly sensitive to oxidative stress that may result protein sulphonic acid formation and cause the destruction of protein structure and activity (Biswas, Chida, & Rahman, 2006). In sum, these studies clearly indicate that different biological thiols may contribute to regulate human health through diverse mechanisms. Thus, CAP, GSH, and CYS in this study are further verified and compared their antimutagenic activities and regulation on activities of antioxidant enzymes in different biological system models.

2. Materials and methods

2.1. Materials

Captopril, glutathione, cysteine and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2-amino-3-methylimidazo [4,5-*f*] quinoline (IQ) was purchased from Waco Chemical Co. (Richmond, VA, USA). All reagents used in the test were of analytical grade. The culture medium was prepared by dissolving 0.8 g of nutrient broth in 100 ml of water. Minimal agar plate was made up by 2% glucose, 0.35 g $NaHNH_4PO_4{\cdot}4H_2O$, 10 g K_2HPO_4, 2 g citric acid monohydrate, 0.2 g $MgSO_4{\cdot}7H_2O$ and 15 g agar in 1000 ml of sterile water. Top agar was prepared by adding 5 g NaCl and 6 g agar in 1000 ml of sterile water.

2.2. Mutagenicity assay

The mutagenicity of biological thiols samples was tested according to the Ames test with a 20 min first incubation at 37 °C (Maron & Ames, 1983). The histidine-requiring strains of *Salmonella typhimurium* TA 98 and TA 100 were purchased from Bioresources Collection and Research Center (Shin-chu, Taiwan). The external metabolic activation system, S9 mix (Organ Teknika Co., Switerland) was prepared from Sprague–Dawley male rats treated with Aroclor 1254. Biological thiols samples (0.1 ml, 5.0–50 mg/ml corresponding to 0.5–5.0 mg/plate, respectively) were added to the overnight cultured *S. typhimurium* TA98 or TA 100 (0.1 ml) and S9 mix (0.5 ml) or 0.1 M phosphate buffer (0.5 ml, pH 7.4) in place of the S9 mix. The entire mixture was incubated at 37 °C for 20 min before molten top agar (2.0 ml) was added and then spread out in a Petri dish containing 20 ml of minimum agar. The mixture was counted after incubating at 37 °C for 48 h. Each sample was assayed in triplicate plates per run, and data prepared are means ± SD of at least two experiments. To examine the toxic effect of biological thiols samples on *S. typhimurium* TA 98 and TA 100, the mixtures after incubation were diluted with 0.1 M phosphate buffer (0.5 ml, pH 7.4), and the diluted mixtures were poured into minimal agar plates that containing $MgCl_2$, KCl, glucose-6-phosphate, and NADP. The plates were incubated at 37 °C for 2 days, and the number of colonies was counted.

2.3. Antimutagenic activity assay

The antimutagenic activity of samples was assayed according to the Ames method except for the addition of mutagen before incubation (Maron & Ames, 1983). Dose of mutagens were selected according previous experiment (Kulwat, Lertprasertsuke, Leechanachai, Kongtawelert, & Vinitketkumnuen, 2005). The mutagens used were IQ (0.1 μg/plate for TA 98 and TA 100, respectively), which required S9 mix for metabolic activation; MNNG (0.5 μg/plate for TA 98 and TA 100, respectively), a direct-acting mutagen. Mutagen (0.1 ml) was added to the mixture of a strain (TA 98 or TA 100), and samples was added with the S9 mix for IQ or with phosphate buffer (0.1 M, pH 7.4) for MNNG. The mutagenicity of each mutagen in the absence of samples is defined as 100%. The number of spontaneous revertants in the absence of mutagens and samples was used as reference. The inhibition (%) of mutagenicity of sample was calculated as following:

$$\text{Inhibition}(\%) = \{1 - [(\text{no. of his}^+\text{revertants in the presence of sample} - \text{no. of spontaneous revertants}) / (\text{no. of his}^+\text{revertants in the absence of sample} - \text{no. of spontaneous revertants})]\} \times 100.$$

2.4. Protective effect on BNL cells viability

BNL cells (ATCC number: TIB-73), an embryonic murine hepatocytes cell line, were purchased from Bioresources Collection and Research Center (Shin-chu, Taiwan) and cultured in DMEM medium containing 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM pyruvate and maintained in humidified 5% CO_2/95% air at 37 °C. After cells were cultured with 1 mM H_2O_2 in the presence of samples or not for 24 h, cell viability was determined by colorimetric measurement of the reduction product of MTT. Briefly, the original medium was removed, then, MTT (final 0.5 mg/ml) were added to each well. After 1 h incubation, the reaction was terminated and the plates were incubated for 30 min to solubilize the formazan dye by addition of dimethyl sulphoxide. The optical density of each well was measured at 570 nm.

2.5. Effect on antioxidant enzyme activities in BNL cells

After BNL cells were cultured in the presence of sample for 24 h, cells (1×10^7 cells) were collected and centrifuged at 1000*g* for 10 min. The pellets were then resuspended in 10 mM phosphate buffer (0.5 ml, pH 7.4), containing 1.5% (w/v) KCl and 1 mM ethylenediaminetetraacetic acid (EDTA), and lysed by three cycles of freezing, thawing and sonication for 10 s at 60 W. The cellular homogenates were centrifuged at 10000*g* for 30 min and the supernatants for the subsequent determination of antioxidant enzyme activities were stored at −80 °C. Intracellular activities of GPX and GR were measured by following the decrease in the absorbance due to oxidation of NADPH. Briefly, in a reaction mixture