Food Chemistry 197 (2016) 829–835

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Protective effects of five allium derived organosulfur compounds against mutation and oxidation

Chih-Kwang Chiu [a], Tai-Yuan Chen [b], Jou-Hsing Lin [c], Chen-Ya Wang [c], Bor-Sen Wang [d,*]

[a] Department of Food Science & Technology, Chia Nan University of Pharmacy and Science, No. 60, Sec. 1, Erren Rd., Tainan, Taiwan, ROC
[b] Department of Food Science, National Taiwan Ocean University, No. 2, Pei-Ning Road, Keelung, Taiwan, ROC
[c] Department of Biotechnology, Chia Nan University of Pharmacy and Science, No. 60, Sec. 1, Erren Rd., Tainan, Taiwan, ROC
[d] Department of Applied Life Science & Health, Chia Nan University of Pharmacy and Science, No. 60, Sec. 1, Erren Rd., Tainan, Taiwan, ROC

ARTICLE INFO

Article history:
Received 2 June 2013
Received in revised form 13 September 2015
Accepted 13 November 2015
Available online 14 November 2015

Keywords:
Organosulfur compounds
Mutation
Cytotoxicity
Antioxidation

ABSTRACT

In this study, we examined the ability of five allium-derived organosulfur compounds to protect cells against mutation and oxidation. The compounds tested were 1-propylmercaptan (PM), dimethyl disulfide (DMDS), diallyl disulfide (DADS), propyl disulfide (PDS), and 2,5-dimethylthiophene (DMT). Our results showed that when used at concentrations of 100–400 μmol/l, the five compounds inhibited the mutagenicity of 4-nitroquinoline-*N*-oxide, a direct mutagen, and benzo[a]pyrene, an indirect mutagen, toward *Salmonella typhimurium* TA 98 and TA 100. Furthermore, at these concentrations, all five of the compounds protected HepG2 cells against *tert*-butyl hydroperoxide-induced oxidative cytotoxicity. The compounds likely enhanced cell viability by suppressing the formation of reactive oxygen species and the depletion of glutathione depletion in cells. DMT and PM inhibited mutation and oxidation to a greater extent than DMDS, DADS, and PDS. These results demonstrate for the first time that DMT and PM can contribute to the antimutagenic and the antioxidative property of *Allium* vegetables.

1. Introduction

DNA damage can increase the risk of cancers, atherosclerosis, neurodegenerative disorders, and chronic lung diseases (Halliwell & Gutteridge, 1999; Rebillard, Lefeuvre-Orfila, Gueritat, & Cillard, 2013). Previous reports have indicated that various environmental mutagens present in damaged DNA and induced mutation through distinct mechanisms (Felton & Knize, 1991). For example, 4-nitroquinoline-N-oxide (4-NQO), a strong and direct mutagen, is a quinoline derivative and a carcinogen that can potently induce intracellular oxidative stress and thereby lead to DNA damage (Arima et al., 2006). Benzo[a]pyrene (BP), an organic tetracyclic hydrocarbon that is generated during the incomplete combustion process, induces carcinogenicity in the lung (Felton & Knize, 1991). The metabolic products of these mutagens can bind to DNA at guanine residues, and the formation of DNA adducts results in an increase in DNA mutation, which eventually enhances the risk of tumor initiation and progression.

The mutagens 4-NQO and BP can also be metabolized into products that induce oxidative stress in cells, which reduces glutathione (GSH) levels, destroys biological molecules such as proteins and lipids, and causes cell damage (Yuan & Kaplowitz, 2009). Oxidative stress has been widely reported to play a key role in the pathogenesis of diverse diseases including cancer and cardiovascular disorders (Wang, Lee, Chen, Yu, & Duh, 2012). Moreover, exposure to various chemicals leads to intracellular oxidative stress, which is caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS). For example, *tert*-butyl hydroperoxide (*t*-BHP), an organic hydroperoxide, can be converted into free radicals by cytochrome P450 enzymes (Minotti, Borrello, Palombini, & Galeotti, 1986). Therefore, *t*-BHP is often used as a model compound to induce oxidative stress in vitro and in vivo studies.

Previous epidemiological studies have indicated that diets rich in phytochemicals potently reduce the risk of chronic age-related diseases including cancers and disorder specific to aging (Zhong, Chiou, Pan, & Shahidi, 2012). Some of the phytochemicals might function as antimutagens and reduce cellular mutation and cancer initiation and progression, whereas others phytochemicals might suppress the production of free radicals to protect DNA against oxidative damage. The potential antimutagenic effects of numerous phytochemicals have been tested using the Ames bacterial assays. For example, catechins in green tea were shown to protect against the formation of carcinogenic intermediates, such as free radicals and electrophiles, during the metabolic activation of

* Corresponding author.
E-mail address: wangbs@mail.cnu.edu.tw (B.-S. Wang).

http://dx.doi.org/10.1016/j.foodchem.2015.11.064
0308-8146/

carcinogens and mutagens (Geetha, Garg, Chopra, & Pal Kaur, 2004). Therefore, phytochemicals likely exert protective effects at least partly by preventing mutagenesis and reducing the activity of free radicals. However, in addition to inhibiting mutation, active phytochemicals offer physiological protection against the induction of oxidative stress in cells (Wang, Tang, Chiu, & Huang, 2012). Consequently, the bifunctional protective properties of naturally occurring phytochemicals have been investigated extensively.

Plants of the species *Allium* have been consumed for many centuries. Among *Allium* species, garlic and onion are two well-known and widely used ingredients in Chinese cooking (Corzo-Martinez, Corzo, & Villamiel, 2007). In addition to the flavor that they provide, garlic and onion have been widely reported to exhibit biological effects that are attributed to their high content of organosulfur compounds (OSCs) (Chu, Wang, & Duh, 2009; Corzo-Martinez et al., 2007). These OSCs, which include diallyl sulfide and diallyl disulfide, partly endow garlic and onion with their unique and characteristic odor and flavor, and also their biological properties (Corzo-Martinez et al., 2007). OSCs originating from garlic inhibit carcinogen activation, boost phase 2 detoxifying processes, cause cell cycle arrest mostly in G2/M phase, and stimulate the mitochondrial apoptotic pathway (Iciek, Kwiecien, & Wlodek, 2009). In this study, we investigated how 4-NQO- and BP-induced mutation and *t*-BHP-induced oxidation were affected by five OSCs commonly detected in Allium species; the compounds tested were a thiol (e.g. 1-propylmercaptan, PM), three disulfides (e.g. dimethyl disulfide, DMDS; diallyl disulfide, DADS; and propyl disulfide, PDS), and a heterocyclic sulfide (e.g. 2,5-dimethylthiophene, DMT).

2. Materials and methods

2.1. Materials

1-Propylmercaptan (PM), dimethyl disulfide (DMDS), diallyl disulfide (DADS) propyl disulfide (PDS), 2,5-dimethylthiophene (DMT), 3-(4,5-dmethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 4-nitroquinoline-N-oxide (4-NQO), benzo[a]pyrene (BP) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) and c hloromethylfluorescein-diacetate (CMF-DA) were purchased from Invitrogen Molecular Probes (Grand Island, NY, USA). Culture medium was prepared by dissolving 0.8 g of nutrient broth in 100 ml of water. The minimal agar plates were 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. Measurement of HepG2 cells viability

HepG2 cells were purchased from Bioresources Collection and Research Center (BCRC, Hsinchu, Taiwan) and cultured in minimum essential medium (MEM) containing 10% fetal bovine serum and maintained in humidified 5% CO_2/95% air at 37 °C. After cells were cultured with samples for 24 h, followed by *t*-BHP (0.2 mmol/l) treatment for 6 h, cell viability was determined by the 3-(4,5-dmethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

2.3. Evaluation of reactive oxygen species (ROS) and glutathione (GSH) in HepG2 cells

Intracellular ROS were detected by means of an oxidation-sensitive fluorescent probe dye, 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) (Invitrogen Molecular Probes, NY, USA). H_2-DCFDA is nonfluorescent until removal of the acetate groups by intracellular esterases and reacts with reactive oxygen species to produce the fluorescent product, dichlorofluorescein (DCF) within the cells. To determine the generation of ROS in HepG2 cells, H_2DCFDA was used because it penetrates cell membranes and is hydrolyzed by intracellular esterase to form dichlorofluorescin (DCF). HepG2 cells were pretreated with samples for 24 h, followed by H_2DCFDA (50 μmol/l) and then *t*-BHP (0.2 mmol/l) was added to the medium. After incubation at 37 °C for 3 h, ROS produced from intracellular stress were detected using a Bio-Tek FLx800 microplate fluorescence reader (Winooski, VT, USA) with excitation and emission wavelengths of 485 and 530 nm, respectively. On the other hand, intracellular GSH levels were determined after staining cell with chloromethylfluorescein-diacetate (CMF-DA) (Invitrogen Molecular Probes, NY, USA) (Wang et al., 2012).

2.4. Evaluation of cytochrome P450 1A1 activity in HepG2 cells

To determine the cytochrome P450 1A1 activity in HepG2 cells, the P450-Glo assay kit was performed according to the manufacturer's protocol (Promega, WI, USA). HepG2 cells were pretreated with the P450 1A1 inducer β-naphthoflavone (10 μmol/l) for 24 h, followed by the samples, which were added directly to the medium. After incubation at 37 °C for 6 h, a luminogenic substrate was added to the culture medium and incubated with cells for 3 h. Then the luminescence signal was detected using a Bio-Tek FLx800 microplate luminescence reader (Winooski, VT, USA) (Westerink & Schoonen, 2007).

2.5. Mutagenicity assay

The mutagenicity of 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 obtained from Taiwan Agricultural Chemicals and Toxic Substances Research Institute (Taichung, Taiwan). The external metabolic activation system, S9 mix (Molecular Toxicology, Inc., Boone, NC, USA) was prepared from Sprague–Dawley male rats treated with Aroclor 1254. Samples (0.1 ml, 20–100 mg/ml corresponding to 2–10 mg/plate) were added to the overnight cultured *S. typhimurium* TA98 or TA 100 (0.1 ml) and S9 mix (0.5 ml) or 0.1 mol/l 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 colonies of bacteria in the Petri dish were counted after incubating at 37 °C for 48 h. The toxic effects of samples on *S. typhimurium* TA 98 and TA 100 was determined as previously described (Wang et al., 2012).

2.6. 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). The concentrations of mutagens were tested as in a previous study. The mutagens used were 4-NQO (0.5 μg/plate), a direct mutagen and BP (5 μg/plate), which required S9 mix for metabolic activation. Mutagen (0.1 ml) was added to the mixture of a strain (TA 98 or TA 100), and samples were added with the S9 mix for BP or with phosphate buffer (0.1 mol/l, pH 7.4) for 4-NQO. 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 the sample was calculated as following: