Differential Effects of Garlic Oil and Its Three Major Organosulfur Components on the Hepatic Detoxification System in Rats

Chih-Chung Wu,[†] Lee-Yan Sheen,[‡] Haw-Wen Chen,[§] Wei-Wen Kuo,[‖] Shun-Jen Tsai,[⊥] and Chong-Kuei Lii*,[§]

Department of Nutrition and Health Science, Fooyin Institute of Technology, 151, Chinghsueh Road, Taliao, Kaohsiung County 831, Taiwan; Department of Nutrition, China Medical College, 91 Hsueh-Shih Road, Taichung 404, Taiwan; Department of Nutrition, Chung Shan Medical University, 110, Chien Kuo N. Road, Taichung 402, Taiwan; Department of Food Health, Chungtai Institute of Health Science and Technology, 11, Po-Tze Lane, Takun, Taichung 406, Taiwan; and Department of Food Science, National Chung-Hsing University, 250, Kuo Kuang Road, Taichung 402, Taiwan

The objective of this study was to compare the modulatory effect of garlic oil and its three organosulfur compounds, diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS), on rat hepatic detoxification enzyme activity, and protein and mRNA expression. Rats were orally administered garlic oil (80 or 200 mg/kg bw), DAS (20 or 80 mg/kg bw), DADS (80 mg/kg bw), or DATS (70 mg/kg bw) three times a week for 6 weeks. Control rats received corn oil. According to the results, garlic oil and DAS in dosages of 200 and 80 mg/kg bw, respectively, significantly increased pentoxyresorufin *O*-dealkylase (PROD) activity as compared with the that of the control rats ($P < 0.05$). In contrast, *N*-nitrosodimethylamine demethylase activity in rats that received DADS and DATS was significantly lower than that in the control rats ($P < 0.05$). Ethoxyresorufin *O*-deethylase and erythromycin demethylase activities were not influenced by garlic oil, DAS, DADS, or DATS. To the phase II enzyme, garlic oil, DADS, and DATS significantly increased the glutathione S-transferase (GST) activity toward ethacrynic aicd ($P < 0.05$). Immunoblot assay showed that the protein contents of cytochrome P450 1A1, 2B1, and 3A1 were increased by garlic oil and each of three allyl sulfides, and the change among the allyl sulfides was in the order of DAS > DADS > DATS. The placental form of GST (PGST) level was also increased by garlic oil and the three allyl sulfides, but the increase among the allyl sulfides was DATS ≅ DADS > DAS. P450 2E1, however, was suppressed by each garlic component. Northern blot results indicated that the changes in P450 1A1, 2B1, 3A1, and PGST mRNA levels by garlic components were similar to those noted in the protein levels. These results indicate that the modulatory effect of garlic oil on hepatic drug-metabolizing enzymes can be attributed to its three major allyl sulfide components DAS, DADS, and DATS. These three allyl sulfides vary in modulatory activity, and this variation is related to the number of sulfur atoms in the molecule.

KEYWORDS: Garlic oil; organosulfur compounds; detoxification system

INTRODUCTION

Garlic (*Allium sativum* L.) is a widely consumed herb in foodstuffs and medicines. Numerous studies have shown that garlic exhibits diverse biological activity, including antitumorigenesis, antiatherosclerosis, and detoxification (*1–4*). The

garlic-rich organosulfur compounds (OSCs) are believed to play key roles in these biological effects. Most garlic OSCs are derived from alliin via the action of alliinase and the following rearrangement (*5*). Even so, the species and quantity of OSCs in different garlic products depend on several factors, such as the method of extraction, temperature, and pH (*6*). Steam distillation is widely used to extract and condense the volatile OSCs in garlic, and the final oily product is called garlic oil.

Drug biotransformation is important to all living organisms in protecting them from environmental toxicant insult. It consists of phase I and phase II enzyme systems. These drug-metabolizing enzymes have been shown to be affected by dietary

* Corresponding author. Tel: 886-4-24730022, ext. 1747. Fax: 886-4-24739030. Email: cklii@csmu.edu.tw.
[†] Department of Nutrition and Health Science, Fooyin Institute of Technology.
[‡] Department of Nutrition, China Medical College.
[§] Department of Nutrition, Chung Shan Medical University.
[‖] Department of Food Health, Chungtai Institute of Health Science and Technology.
[⊥] Department of Food Science, National Chung-Hsing University.

Garlic and Detoxification Enzymes

J. Agric. Food Chem., Vol. 50, No. 2, 2002 **379**

nutritional, as well as nonnutritional, factors (*7*). Cytochrome P450 enzymes (P450s), the most important phase I enzymes, catalyze the biotransformation of many xenobiotics and endogenous compounds (*8*). At least 14 gene families have been identified in mammals (*9*), many of which are selectively enhanced or suppressed by a variety of chemicals, including numerous garlic components (*10*, *11*). For instance, diallyl sulfide (DAS) suppressed *N*-nitrosodimethylamine demethylase (NDMAD) activity and P450 2E1 protein expression (*12*, *13*), whereas rat hepatic pentoxyresorufin *O*-dealkylase (PROD) activity and P450 2B1 protein content were induced (*13*−*15*). Although the actual mechanism is not clear yet, the binding of toxins through the sulfhydryl group of OSCs has been proposed (*16*).

Glutathione S-transferase (GST), a phase II drug-metabolizing enzyme, belongs to a family of multifunctional proteins which detoxifies a wide variety of electrophilic xenobiotics by catalyzing their conjugation to glutathione and reduces many organic hydroperoxides into alcohols through its expression of selenium independent glutathione peroxidase activity (*17*). On the basis of amino acid sequence homologies and antibody cross-reactivity, cytosolic GST is grouped into six classes (*18*). Among these GST isozymes, the placental form of GST (PGST) has attracted particular interest because of its relationship with the formation of human ovarian (*19*) and colorectal cancers (*20*). Modulation of PGST activity and protein expression by garlic components has been noted in various tissues in rats and mice (*15*, *21*).

By using gas chromatography, more than 20 OSCs have been identified in garlic oil (*22*). Among these OSCs, DAS, diallyl disulfide (DADS), and diallyl trisulfide (DATS), which differ in their number of sulfur atoms, are the three major constituents. Although the garlic OSC effect on the drug-metabolizing system has attracted a great deal of investigation, DAS and DADS are two key components that have been extensively studied. The study of DATS, however, is limited. It is also interesting to examine whether garlic oil modulation of hepatic drug-metabolizing enzymes can be attributed to the combined effect of DAS, DADS, and DATS. In this study, rats were administered garlic oil or DAS, DADS, or DATS based on their amount in garlic oil, and the effects on the hepatic PGST and P450 activities, protein, and mRNA expressions were investigated.

MATERIALS AND METHODS

Materials. DAS, DADS, and DATS were purchased from Fluka Chemical Co. (Buchs, Switzerland), Tokyo Kasei Chemical Co. (Tokyo, Japan), and LKT Laboratories Inc. (St. Paul, MN), respectively. Fresh garlic was purchased from the local market, and garlic oil was prepared by steam distillation (*23*). The constituent profile of the garlic oil was analyzed and identified using a GC−MS system (G1800 GCD, Hewlett-Packard, Palo Alto, CA). The volatile constituents include DAS (10%), DADS (39%), DATS (35%), and other minor components. The monoclonal antibody against cytochrome P450 1A1 was obtained from GENTEST Co. (Woburn, MA), and polyclonal antibodies against cytochrome P450 2B1, 2E1, or 3A1 were obtained from Chemicon International (Temecular, CA). The PGST antiserum was purchased from Biotrin Co. (Dublin, Ireland).

Animals and Treatments. Four-week-old male Sprague−Dawley rats were purchased from the National Animal Breeding and Research Center (Taipei, Taiwan). After one week of acclimation, rats were randomly assigned to each experimental group by weight and housed with a 12-hr light cycle. Animals had free access to water and AIN-76A diet. The treated dose of garlic oil was either 80 or 200 mg/kg bw. The dosages of DAS, DADS, and DATS were estimated on the basis of their amount in 200 mg of garlic oil and were 20, 80, and 70 mg/kg bw, respectively. An additional 80 mg/kg bw of DAS, close to

the DADS and DATS dosage was also tested in this study. All garlic components were diluted in corn oil and administered by oral intubation three times (Monday, Wednesday, and Friday mornings) each week for 6 weeks. Rats treated with 1 mL/kg bw corn oil alone were regarded as the control.

Rats were fasted overnight and sacrificed by carbon dioxide euthanasia. Fresh livers were removed, minced, and quickly freeze-clamped in liquid nitrogen and stored at −80 °C.

Cytochrome P-450 and GST Activity Assays. Livers were homogenized in 4× of a buffer (pH 7.4) containing 10 mM potassium phosphate and 1.15% KCl, and centrifuged at 10000*g* for 30 min at 4 °C. The resultant supernatants were further ultracentrifuged at 105000*g* for 1 h, and the cytosolic fractions were stored at −80 °C until analysis. The microsomal pellets were resuspended in 50 mM potassium phosphate/1 mM EDTA buffer (pH 7.6) for cytochrome P-450 activity and immunoblot assays. The ethoxyresorufin *O*-deethylase (EROD) activity was measured with a spectrophotometer (U2000, Hitachi, Tokyo, Japan) using a previously described method (*24*). The activity of *N*-nitrosodimethylamine demethylase (NDMAD) and erythromycine demethylase (EMD) were determined by the method of Nash (*25*). 7-Pentoxyresorufin *O*-dealkylase (PROD) activity was measured with fluorescence spectrophotometer (F4500, Hitachi, Tokyo, Japan) as described by Lubet et al. (*26*). Activity of PGST was determined according to the method of Habig et al. (*27*) using ethacrynic acid as the substrate.

SDS−Polyacrylamide Gel Electrophoresis (SDS−PAGE) and Western Blot Analysis. The amounts of cytosolic PGST and microsomal cytochrome P450s were determined by SDS−PAGE and immunoblot assay. Equal amounts of proteins were first electrophoresized in 10% polyacrylamide gels. Proteins separated on SDS−polyacrylamide gels were then transferred to polyvinylidene difluoride membranes. Membranes were incubated at 4 °C overnight with 5% skim milk to block the nonspecific binding, and this was followed by a 30-min incubation at 37 °C with PGST, or P450 1A1, 2B1, 2E1, or 3A1 antiserums. Peroxidase-conjugated secondary antibody and H_2O_2 and tetrachloride diaminobenzidine were used to detect the immunoreactive bands.

Northern Blot Analysis. Total RNA was extracted from 40 mg of fresh liver tissues by homogenizing the liver in 1 mL of Trizol reagent using a Teflon homogenizer. The homogenates were allowed to react at room temperature for 5 min and 0.2 mL of chloroform was then added followed by incubation for an additional 3 min. While the homogenates were centrifuging at 12000*g* for 15 min, RNA in the aqueous phase was precipitated by adding 0.5 mL isopropyl alcohol. The RNA pellet was then prepared by centrifuging at 12000*g* for 10 min, and it was stored in 75% ice-cold ethanol.

For Northern blot analysis, 20 μg of each RNA sample was electrophoretically separated in 1% agarose gel containing 6% form-aldehyde and transferred to HYBond membranes as previously described (*23*). The membranes were prehybridized for 2 h at 42 °C in a solution containing 10× Denhardt's reagent (0.2% Ficoll, 0.2% polyvinylprolidone, and 0.2% bovine serum albumin), 5× SSPE (750 mM NaCl, 50 mM NaH_2PO_4, and 5 mM EDTA), 2% SDS, 50% formamide, and 100 μg/mL of single-strand sheared salmon sperm DNA. The membranes were then hybridized in the same solution with ^{32}P-labeled cDNA probes at 42 °C overnight. cDNA probes for PGST and P450s were generated by PT−PCR as described (*27*). Two pairs of oligonucleotide primers were designed on the basis of the published sequence and were as follows: 1A1 (forward, 5′-CTGCCTTGGAT-TCTGGGTGGTT-3′; backward, 5′-CAAAGGATGAATGTCGGAAG-GT-3′); 2B1 (forward, 5′-GGATGGGAAAGAGGAGTGTGGA-3; backward, 5-CTGGAGGATGGTGGTGAAGAAG-3′); 3A1 (forward, 5′-TTGCCATCACGCACACAGAAT-3′; backward, 5′-ATGCTGC-CCTTGTTCTCCTTGC-3′); and PGST (forward, 5′-TTCAAGGCTC-GCTCAAGTCCAC-3′; backward, 5′-CTTGATCTTGGGGCGGGCA-CTG-3′). Autoradiography was performed by exposing the membrane to Kodak SuperRx X-ray film. For rehybridization with the other cDNA probes, membranes were deprobed by washing them with boiling 0.1% SDS. The bands on the X-ray film were quantitated with an AlphaImager2000 (Alpha Innotech Co., San Leandro, CA).

380 *J. Agric. Food Chem.,* Vol. 50, No. 2, 2002

Wu et al.

Table 1. Effects of Garlic Oil and Allyl Sulfides on Hepatic Ethoxyresorufin *O*-deethylase (EROD), *N*-nitrosodimethylamine *N*-demethylase (NDMAD), Pentoxyresorufin *O*-demethylase (PROD), and Erythromycin Demethylase (EMD) Activities[a]

treatment	dose mg/kg	EROD pmol/min/mg protein	EMD nmol/min/mg potein	PROD pmol/min/mg protein	NDMAD nmol/min/mg protein
control	- -	13.4 ± 2.5[*,†]	0.36 ± 0.15	8.4 ± 2.5[‡]	0.81 ± 0.18[*]
GO	80	18.6 ± 2.9[*,†]	0.64 ± 0.29	11.6 ± 5.1[†,‡]	0.52 ± 018[*,†]
GO	200	18.6 ± 2.9[*,†]	0.67 ± 0.20	15.9 ± 1.4[*,†]	0.56 ± 0.15[*,†]
DAS	20	22.3 ± 3.6[*,†]	0.67 ± 0.27	10.6 ± 1.8[†,‡]	0.61 ± 0.28[*,†]
DAS	80	24.7 ± 7.2[*]	0.80 ± 0.35	22.3 ± 5.9[*]	0.57 ± 0.28[*,†]
DADS	80	19.6 ± 8.4[*,†]	0.56 ± 0.28	9.6 ± 2.7[†,‡]	0.37 ± 0.05[†]
DATS	70	12.0 ± 2.8[†]	0.62 ± 0.26	9.2 ± 1.4[†,‡]	0.47 ± 0.06[†]

[a] Rats were orally administered with various doses of garlic oil and three allyl sulfides for 6 weeks. Data are expressed as means± SD (*n* = 4 − 6). GO, garlic oil; DAS, diallyl sulfide; DADS, diallyl disulfide; DATS, diallyl trisulfide. [*,†,‡]Treatment means not sharing the same symbols differ significantly (*P* < 0.05) by Tukey's comparison.

Table 2. Effects of Garlic Oil and Allyl Sulfides on Hepatic Glutathione S-Transferase (GST) Activities[a]

treatment	dose mg/kg	GST nmol/min/mg protein
control	- - - - -	375 ± 70[‡]
GO	80	496 ± 51[*,†]
GO	200	533 ± 51[*]
DAS	20	382 ± 68[‡]
DAS	80	436 ± 51[*,†]
DADS	80	555 ± 104[*]
DATS	70	532 ± 77[*]

[a] Rats were orally administered with various doses of garlic oil and three allyl sulfides for 6 weeks. Data are expressed as means± SD (*n* = 4–6). GO, garlic oil; DAS, diallyl sulfide; DADS, diallyl disulfide; DATS, diallyl trisulfide. [*,†,‡]Treatment means not sharing the same symbols differ significantly by Tukey's comparison (*P* < 0.05).

Statistical Analysis. Statistical analyses were performed using ANOVA (SAS Institute Inc., Cary, NC). Tukey's multiple comparison was used to determine significant differences among the group means (*P* < 0.05).

RESULTS

Drug-Metabolizing Enzyme Activities. After 6 weeks of treatment, the growth rate of the rats did not change from each garlic component as compared with the controls (data not shown). The effects of garlic oil and three allyl sulfides on the hepatic phase I drug-metabolizing enzyme activities are shown in Table 1. No change in the EROD and EMD activity was noted in rats administered either with garlic oil or each allyl sulfide as compared with that in the control rats. Rats that received high doses of garlic oil (200 mg/kg) or DAS (80 mg/kg) had higher PROD activities than the controls (*P* < 0.05). With the exception of the 200 mg/kg garlic oil group, 80 mg/kg DAS caused significantly higher PROD activity than the other garlic groups (*P* < 0.05). In contrast to the increase of PROD activity, NDMAD activity in rats that received DADS and DATS was significantly lower than that in the control rats (*P* < 0.05).

To measure the PGST activity, ethacrynic acid was used as the substrate for the enzyme activity assay instead of 1-chloro-2,4-dinitrobenzene. As the results indicated, garlic oil, either in a dose of 80 or 200 mg/kg bw, DAD,S or DATS significantly increased hepatic PGST activity as compared to that of the control rats (*P* < 0.05)(Table 2). DAS, either in low or high doses, however, had no influence on this phase II enzyme activity.

Cytochrome P450 1A1, 2B1, 2E1, 3A1, and PGST Protein Levels. Immunoblot assay showed that garlic oil and its three allyl sulfide components actively modulated four hepatic
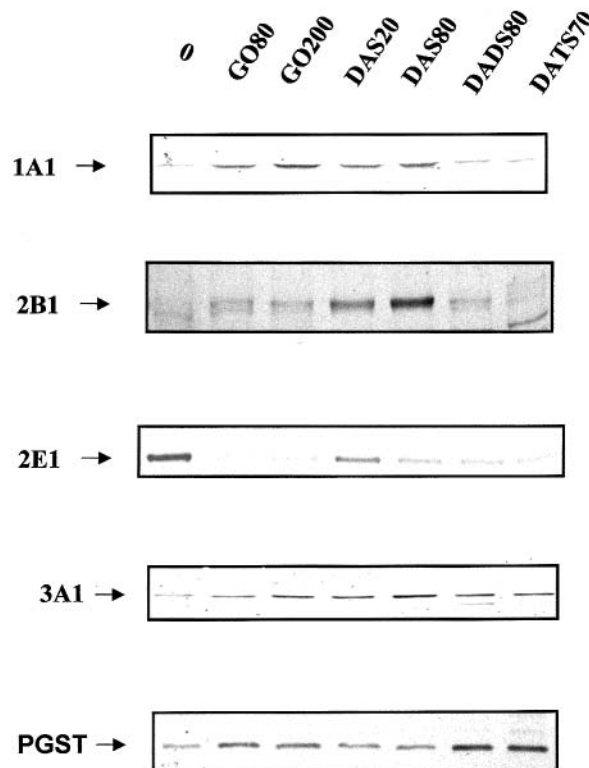

Figure 1. Immunoblot analysis of P450s and PGST in rats treated with garlic oil (GO) or its three major allyl sulfides. Control rats received corn oil alone. Proteins were separated on 10% SDS–polyacrylamide gels and were electrophoretically transferred to poly(vinylidene difluoride) membranes. The amount of proteins in each lane for P450s and PGST immunostaining was 6 and 3 μg, respectively.

cytochrome P450s and PGST protein expression but in different manners (Figure 1). P450 1A1 and 2B1 levels were higher in each garlic-treated group than in the control. A dose-dependent increase was also noted in rats dosed with garlic oil and DAS. Among allyl sulfides, DAS showed a greater induction than DADS and DATS. Even the DAS in a dose of 20 mg/kg resulted in higher levels of P450 1A1 and 2B1 than DADS and DATS. DADS had greater induction than DATS. Similar induction was also noted in the P450 3A1. Rats dosed with 80 mg/kg DAS had the highest protein level. In contrast to the induction, cytochrome P450 2E1 expression was suppressed by either garlic oil or each of the three allyl sulfides. P450 2E1 levels in DADS and DATS groups were similar to that in the 80 mg/kg DAS group.

Regarding the phase II drug-metabolizing enzyme, PGST expression in rat livers was increased by garlic oil and its three
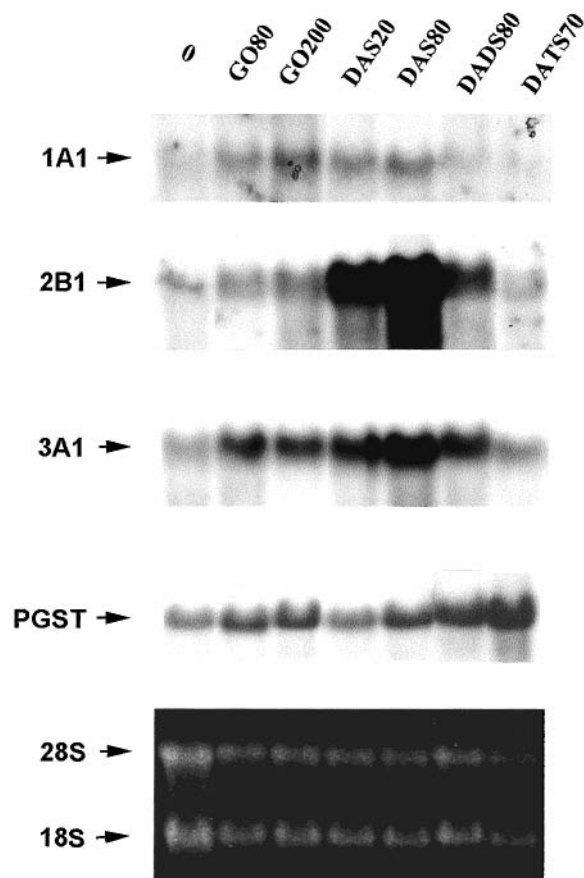
Garlic and Detoxification Enzymes

J. Agric. Food Chem., Vol. 50, No. 2, 2002   **381**

Figure 2. Contents of hepatic P450 1A1, 2B1, 2E1, and 3A1 and PGST mRNA in rats orally administered with garlic oil, diallyl sulfide (DAS), diallyl disulfide (DADS), or diallyl trisulfide (DATS). Total RNA was extracted using Trizol as described in Materials and Methods. A 20-μg aliquot of RNA of each sample was subjected to Northern blot analysis. The membrane was first hybridized with a P450 2B1 cDNA and then rehybridized in an order with 1A1, 3A1, and PGST cDNA.

major allyl sulfide components. The potency of induction among allyl sulfides, however, was different from that noted on cytochrome P450 1A1, 2B1, and 3A1. For this phase II detoxifying enzyme, rats dosed with DADS or DATS showed a greater induction than those with either 20 or 80 mg/kg bw DAS. The PGST level was similar between DADS and DATS.

mRNA Expression. Northern blot assay revealed that cytochrome P450 1A1, 2B1, and 3A1 and PGST mRNA levels were accompanied with the changes in protein levels (Figure 2). As noted, garlic oil and DAS dose-dependently increased mRNA contents of all four drug-metabolizing enzymes. For three P450s, DAS had the greater potency in induction of mRNA than DADS, and DADS was greater than DATS. Even DAS at the dose of 20 mg/kg bw caused higher mRNA levels as compared with 80 mg/kg bw DADS and 70 mg/kg bw DATS. As examined, the extent of DAS induction of P450 mRNA levels, 2B1 increased much more than that noted in 1A1 and 3A1. As quantitated by densitometry, the induction of P450 1A1, 2B1, and 3A1 by 80 mg/kg DAS were 3.1, 18.8, and 9.3-fold, respectively, as compared to those of the control rats. The increase of mRNA content by garlic oil was less than that of DAS but was higher than DADS and DATS. Increase of PGST mRNA level was also noted in rats treated with garlic oil or each of the allyl sulfides as compared with those of the control rats. Among allyl sulfides, similar to the changes noted on

protein content, DADS or DATS had higher PGST mRNA expression than DAS.

DISCUSSION

In various animal models, garlic has been demonstrated to be effective in suppressing tumor formation (*2, 3, 28, 29*). Epidemological evidence also supports that garlic consumption is inversely correlated to the incidence of stomach, breast, and colon cancer in China, Italy, France, and the United States (*30–33*). Several mechanisms have been proposed to participate in the antitumorigenic effects of garlic, including an increase in antioxidant defense activity and the modulation of immune responses, such as splenocyte proliferation, cytokine secretion, and natural killer cell activities (*34, 35*). In addition, modulation on the drug-metabolizing enzyme activity by garlic and its active components has also been proposed (*11*).

Although biotransformation of xenobiotics is regarded as a detoxification process, this is not always the case. Some carcinogens may sometimes be activated and are more toxic than the parent compounds. The balance between detoxification and bioactivation determines the net effect (*36*). The inhibitory effect of DAS on cytochrome P450 2E1, which catalyzes the oxidation of many volatile environmental carcinogens (*37*), has been implicated in its anticarcinogenic capability. Instead, the increase in cytochrome P450 2B1 activity, which oxidizes foreign lipophilic compounds and is responsible for the introduction of a polar group into the substrate molecule, by DAS enhances the detoxification in livers (*14*). The results of this study showed that the volatile OSC-rich garlic oil is effective in modulating both phase I and II drug-metabolizing enzyme expression in the transcriptional stage. Whether the modulation is induction or suppression, it depends on the enzyme examined. With the exception of the inhibition of P450 2E1 expression, P450 1A1, 2B1, and 3A1, and also the PGST protein and mRNA, levels were increased by garlic oil. On the basis of the results noted in the allyl sulfide groups, results revealed that the effect of garlic oil on these drug-metabolizing enzymes is related to DAS, DADS, and DATS, which account for 80–85% of the total volatile OSCs in garlic oil. However, the effect of garlic oil on P450s and PGST activities and expression was less effective as compared with the quantitatively combined effects of each individual allyl sulfide. This implicates that the existence of chemical interactions among DAS, DADS, and DATS, and/or the effect of other minor volatile allyl sulfides, in garlic oil on P450s and PGST activities and expression is possible.

In this study, the sulfur atom numbers of allyl sulfides was demonstrated to correlate with the potency in modulating the drug-metabolizing enzyme expression. For P450 1A1, 2B1, and 3A1, protein and mRNA levels were inversely correlated to the number of sulfur atoms and were in the order of DAS > DADS > DATS. In contrast, such a structure–function relationship for PGST expression was reversed. For this phase II enzyme, DADS and DATS showed greater potency in the induction than DAS. The differential effect of DAS, DADS, and DATS on drug-metabolizing enzymes accounts, at least in part, for their difference in anticarcinogenic activity (*3, 29*). Similar structure–function relationship of garlic allyl sulfides has also been reported on the number of allyl groups on the suppression of colon cancer formation in mice (*3, 29*) and the number of sulfur atoms on the hepatic antioxidant enzyme activity in rats (*38*). It is not clear at present what causes such a differential structure–function relationship in modulating the P450s and PGST, and this requires further study. Different regulatory

382 *J. Agric. Food Chem.,* Vol. 50, No. 2, 2002

Wu et al.

pathways for P450s and PGST and/or varied binding affinity of allyl sulfides and their metabolic products may explain in part this discrepancy.

DAS in rat livers was metabolized into diallyl sulfoxide and diallyl sulfone. Diallyl sulfoxide has been shown to be responsible for the increase in the P450 2B1/2 mRNA and protein expression, and, thus, the activity of PROD (*14*). Diallyl sulfone has also been thought to participate in the inhibition of NDMAD activity and the decrease in P450 2E1 level in the hepatic microsomes of rats treated with DAS (*12*). DADS was metabolized into allyl mercaptan rather than diallyl sulfone (*39*). Allyl mercaptan inhibition of P450 2E1 at the posttranscriptional stage has been reported (*40*). In addition to this evidence, more investigations are required before drawing a solid conclusion on the biological roles of garlic metabolites. For instance, the possible roles of allyl sulfides and their metabolites in the degradation of specific P450 mRNA, in the translation process, and in the P450 degradation through protein turnover or by suicide inhibition, remain for further study.

Although P450 genes share similar regulatory elements, their expression pattern is complex and depends on several factors, such as the gender, age, and cell type (*41*). At present, little is known about the transcriptional regulation of 2B genes by drugs in rats, and the presence of specific receptors for such drugs has not yet been identified. Instead, the regulation of P450 1A is much better understood. Enhancement of the P450 1A expression by polycyclic aromatic hydrocarbons is now know to occur through the Ah receptor, in which the ligand−receptor complex translocates into the nucleus and binds to xenobiotic-responsive elements (*42*). It is still not clear what mechanism causes DAS and other garlic components to modulate P450 gene expression. In this study, cytochrome P450 1A1, 2B1, and 3A1 levels were co-modulated by DAS, however, the extent of increase was dramatically different and was in the order of 2B1 > 3A1 > 1A1. This result indicates that garlic allyl sulfides simultaneously modulate a couple of P450 genes, but with variable efficacy.

In conclusion, the modulation of garlic oil on the hepatic drug-metabolizing system could be explained by the combined effect of DAS, DADS, and DATS. These three allyl sulfides differentially modulate the hepatic P450s and PGST mRNA and protein levels, and the potency is related to the number of sulfur atoms in the molecule.

ABBREVIATIONS USED

GO, garlic oil; DAS, diallyl sulfide; DADS, diallyl disulfide; DATS, diallyl trisulfide; P450, cytochrome P450; EMD, erythromycin demethylase; EROD, ethoxyresorufin *O*-deethylase; GST, glutathione S-transferase; NDMAD, *N*-nitrosodimethylamine demethylase; OSCs, organosulfur compounds; PGST, placental form of glutathione S-transferase; PROD, pentoxyresorufin *O*-dealkylase.

LITERATURE CITED

(1) Ali, M.; Al-Qattan, K. K.; Al-Enezi, F.; Khanafer, R. M.; Mustafa, T. Effect of allicin from garlic powder on serum lipids and blood pressure in rats fed with a high cholesterol diet. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2000**, *62*, 253−259.

(2) Fukushima, S.; Takada, N.; Hori, T.; Wanibuchi, H. Cancer prevention by organosulfur compounds from garlic and onion. *J. Cell. Biochem.* (*suppl.*) **1997**, *27*, 100−105.

(3) Sumiyoshi, H.; Wargovich, M. J. Chemoprevention of 1,2-dimethylhydrazine-induced colon cancer in mice by naturally occurring organosulfur compounds. *Cancer Res.* **1990**, *50*, 5084−5087.

(4) Superko, H. R.; Krauss, R. M. Garlic powder effect on plasma lipids, postprandial lipemia, low-density lipoprotein particle size, high-density lipoprotein subclass distribution and lipoprotein-(a). *J. Am. Coll. Cardiol.* **2000**, *35*, 321−326.

(5) Stoll, V.; Seebeck, E. Allium compounds. I. Alliin, the true mother compound of garlic oil. *Helv. Chim. Acta* **1948**, *31*, 189−210.

(6) Yu, T.-H., Wu, C.-M.; Chen, S. Y. Effects of pH adjustment and heat treatment on the stability and the formation of volatile compounds of garlic. *J. Agric. Food Chem.* **1989**, *37*, 730−734.

(7) Guengerich, F. P. Influence of nutrients and other dietary materials on cytochrome P-450 enzymes. *Am. J. Clin. Nutr.* **1995**, *61* (*suppl.*), 651S−658S.

(8) Guengerich, F. P. Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Res.* **1988**, *48*, 2946−2954.

(9) Nelson, D. R.; Kamataki, T.; Waxman, D. J.; Guengerich, F. P.; Estabrook, R. W.; Feyereisen, R.; Gonzalez, F. J.; Coon, M. J.; Gunsalus, I. C.; Gotoh, O. The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA Cell Biol.* **1993**, *12*, 1−51.

(10) Reicks, M. M.; Crankshaw, D. L. Modulation of rat hepatic cytochrome P-450 activity by garlic organosulfur compounds. *Nutr. Cancer* **1996**, *25*, 241−248.

(11) Yang, C. S.; Wang, Z. Y.; Hong, J. Y. Inhibition of tumorigenesis by chemicals from garlic and tea. *Adv. Exp. Med. Biol.* **1994**, *354*, 113−122.

(12) Brady, J. F.; Ishizaki, H.; Fukuto, J. M.; Lin, M. C.; Fadel, A.; Gapac, J. M.; Yang, C. S. Inhibition of cytochrome P-450 2E1 by diallyl sulfide and its metabolites. *Chem. Res. Toxicol.* **1991**, *4*, 642−647.

(13) Haber, D.; Siess, M, H.; Canivenc-Lavier, M. C.; Le Bon, A. M.; Suschetet, M. Differential effects of dietary diallyl sulfide and diallyl disulfide on rat intestinal and hepatic drug-metabolizing enzymes. *J. Toxicol. Environ. Health* **1995**, *44*, 423−434.

(14) Pan, J.; Hong, J. Y.; Ma, B. L.; Ning, S. M.; Paranawithana, S. R.; Yang, C. S. Transcriptional activation of cytochrome P450 2B1/2 genes in rat liver by diallyl sulfide, a compound derived from garlic. *Arch. Biochem. Biophys.* **1993**, *302*, 337−342.

(15) Sheen, L. Y.; Chen, H. W.; Kung, Y. L.; Liu, C. T.; Lii, C. K. Effects of garlic oil and its organosulfur compounds on the activities of hepatic drug-metabolizing and antioxidant enzymes in rats fed high- and low-fat diets. *Nutr. Cancer* **1999**, *35*, 160−166.

(16) Shenoy, N. R.; Choughuley, A. S. Inhibitory effect of diet related sulfhydryl compounds on the formation of carcinogenic nitrosamines. *Cancer Lett.* **1992**, *65*, 227−232.

(17) Jakoby, W. B. The glutathione S-transferases: a group of multifunctional detoxification proteins. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1978**, *46*, 383−414.

(18) van Iersel, M. L. P. S.; Verhagen, H.; van Bladeren, P. J. The role of biotransformation in dietary (anti)carcinogenesis. *Mutat. Res.* **1999**, *443*, 259−270.

(19) Hamada, S.; Kamada, M.; Furumoto, H.; Hirao, T.; Aono, T. Expression of glutathione S-transferase-pi in human ovarian cancer as an indicator of resistance to chemotherapy. *Gynecol. Oncol.* **1994**, *52*, 313−319.

(20) Mulder, T. P.; Verspaget, H. W.; Sier, C. F.; Roelofs, H. M.; Ganesh, S.; Griffioen, G.; Peters, W. H. Glutathione S-transferase-pi in colorectal tumors is predictive for overall survival. *Cancer Res.* **1995**, *55*, 2696−2702.

(21) Hu, X.; Singh, S. V. Glutathione S-transferases of female A/J mouse lung and their induction by anticarcinogenic organosulfides from garlic. *Arch. Biochem. Biophys.* **1997**, *340*, 279−286.

Garlic and Detoxification Enzymes

J. Agric. Food Chem., Vol. 50, No. 2, 2002 **383**

(22) Yu, T.-H.; Wu, C.-M.; Liou Y.-C. Volatile compounds from garlic. *J. Agric. Food Chem.* **1989**, *37*, 725−730.

(23) Chen, H. W.; Yang, J. J.; Tsai, C. W.; Wu, J. J.; Sheen, L. Y.; Ou, C. C.; Lii, C. K. Dietary fat and garlic oil independently regulate hepatic cytochrome P450 2B1 and the placental form of glutathione S-transferase expression in rats. *J. Nutr.* **2001**, *131*, 1438−1443.

(24) Pohl, R. J.; Fouts, J. R. A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Anal. Biochem.* **1980**, *107*, 150−155.

(25) Nash, T. The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.* **1953**, *55*, 416−421.

(26) Lubet, R. A.; Mayer, R. T.; Cameron, J. W.; Nims, R. W.; Burke, M. D.; Wolff, T. Guengerich, F. P. Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. *Arch. Biochem. Biophys.* **1985**, *238*, 43−48.

(27) Habig, W. H.; Pabst, M. J.; Jakoby, W. B. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **1974**, *249*, 7130−7139.

(28) Hu, P. J.; Wargovich, M. J. Effect of diallyl sulfide on MNNG-induced nuclear aberrations and ornithine decarboxylase activity in the glandular stomach mucosa of the Wistar rat. *Cancer Lett.* **1989**, *47*, 153−158.

(29) Srivastava, S. K.; Hu, X.; Xia, H.; Zaren, H. A.; Chatterjee; M. L.; Agarwal, R.; Singh, S. V. Mechanism of differential efficacy of garlic organosulfides in preventing benzo[*a*]pyrene-induced cancer in mice. *Cancer Lett.* **1997**, *118*, 61−67.

(30) Buiatti, E.; Palli, D.; Decarli, A.; Amadori, D.; Avellini, C.; Bianchi, S.; Biserni, R.; Cipriani, F.; Cocco, P.; Giacosa, A. A case-control study of gastric cancer and diet in Italy. *Intl. J. Cancer* **1989**, *44*, 611−616.

(31) Challier, B.; Perarnau, J. M.; Viel, J. F. Garlic, onion and cereal fiber as protective factors for breast cancer: a French case-control study. *Eur. J. Epidemiol.* **1997**, *14*, 737−747.

(32) Steinmetz, K. A.; Kushi, L. H.; Bostick, R. M.; Folsom, A. R.; Potter, J. D. Vegetables, fruit, and colon cancer in the Iowa Women's Health Study. *Am. J. Epidemiol.* **1994**, *139*, 1−15.

(33) You, W. C.; Blot, W. J.; Chang, Y. S.; Ershow, A.; Yang, Z. T.; An, Q.; Henderson, B. E.; Fraumeni, J. F., Jr.; Wang, T. G. Allium vegetables and reduced risk of stomach cancer. *J. Natl. Cancer Inst.* **1989**, *81*, 162−164.

(34) Colic, M.; Savic, M. Garlic extracts stimulate proliferation of rat lymphocytes in vitro by increasing IL-2 and IL-4 production. *Immunopharmacol. Immunotoxicol.* **2000**, *22*, 163−181.

(35) Salman, H.; Bergman, M.; Bessler, H.; Punsky, I.; Djaldetti, M. Effect of a garlic derivative (alliin) on peripheral blood cell immune responses. *Intl. J. Immunopharmacol.* **1999**, *21*, 589−597.

(36) Timbrell, J. A. Toxication versus detoxication. In Principles of Biochemical Toxicology; Timbrell, J. A., Ed.; Taylor and Francis Press: London, 1991; pp 121−123.

(37) Guengerich, F. P.; Kim, D. H.; Iwasaki, M. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem. Res. Toxicol.* **1991**, *4*, 168−179.

(38) Wu, C. C.; Sheen, L. Y.; Chen, H.-W.; Tsai, S.-J.; Lii, C.-K. Effects of organosulfur compounds from garlic oil on the antioxidation system in rat liver and red blood cells. *Food Chem. Toxicol.* **2001**, *39*, 563−569.

(39) Egen-Schwind, C.; Eckard, R.; Kemper, F. H. Metabolism of garlic constituents in the isolated perfused rat liver. *Planta Med.* **1992**, *58*, 301−305.

(40) Kwak, M. K.; Kim, S. G.; Kim, N. D. Effects of garlic oil on rat hepatic P450 2E1 expression. *Xenobiotica* **1995**, *25*, 1021−1029.

(41) Lewis, D. F. A. *Cytochrome P450: Structure, Function and Metabolism*; Taylor and Francis: London, UK, 1996; pp 169−208.

(42) Whitlock, J. P., Jr. The regulation of gene expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Pharmacol. Rev.* **1987**, *39*, 147−161.