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The Antioxidant Properties of Garlic Compounds: Allyl Cysteine, Alliin, Allicin, and Allyl Disulfide

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ABSTRACT Garlic and garlic extracts, through their antioxidant activities, have been reported to provide protection against free radical damage in the body. This study investigated antioxidant properties of garlic compounds representing the four main chemical classes, alliin, allyl cysteine, allyl disulfide, and allicin, prepared by chemical synthesis or purification. Alliin scavenged superoxide, while allyl cysteine and allyl disulfide did not react with superoxide. Allicin suppressed the formation of superoxide by the xanthine/xanthine oxidase system, probably *via* a thiol exchange mechanism. Alliin, allyl cysteine, and allyl disulfide all scavenged hydroxyl radicals; the rate constants calculated based on deoxyribose competitive assay were 1.4–1.7 $\times$ 10^{10}, 2.1–2.2 $\times$ 10^{9}, and 0.7–1.5 $\times$ 10^{10} M^{-1} second^{-1}, respectively. Contrary to previous reports, allicin did not exhibit hydroxyl radical scavenging activity in this study. Alliin, allicin, and allyl cysteine did not prevent induced microsomal lipid peroxidation, but both alliin and allyl cysteine were hydroxyl scavengers, and allyl disulfide was a lipid peroxidation terminator. In summary, our findings indicated that allyl disulfide, alliin, allicin, and allyl cysteine exhibit different patterns of antioxidant activities as protective compounds against free radical damage.

KEY WORDS: • ***alliin*** • ***allicin*** • ***allyl cysteine*** • ***allyl disulfide*** • ***antioxidant*** • ***garlic***

INTRODUCTION

FREE RADICALS HAVE BEEN IMPLICATED in various pathophysiological conditions, which include aging, toxicity, inflammation, tumor promotion, and atherosclerosis.[1–4] These radicals may be derived from metabolism of xenobiotics, as by-products of the normal metabolic pathways or originating from the environment.[4,5] These highly reactive radicals can react with biological molecules such as membranes and DNA, which may ultimately lead to cell death. However, they are also formed endogenously to perform biological functions. For example, some cell types such as macrophages and leukocytes generate superoxide anions during an oxidative burst, which are subsequently converted to hypochlorous acid by myloperoxidase as the toxicant to defend the body from invading microorganisms.[6–8] In addition, superoxide anions released by fibroblasts have been suggested to play a role in cellular or subcellular function.[9–11] Excessive oxidant damage is prevented in the body by various enzymatic and non-enzymatic antioxidant defense systems such as superoxide dismutase, catalase, glutathione peroxidase, glutathione *S*-transferase, uric acid, ascorbic acid, ceruplasmin, glutathione, etc.[2,3,12,13] If the defense system is unable to cope with the insulting radicals, the unreacted radicals may react with the cellular components, leading ultimately to the peroxidation of membrane lipids, enzyme deactivation, damage of DNA bases, and cell death.[4,14,15] For this reason, it is important to protect the body from excessive radical damage.[4,12]

Garlic, a common culinary ingredient has been used for medicinal purposes from ancient times.[16,17] It exerts various biological effects such as lowering cholesterol levels,[18–21] inhibiting platelet aggregation[22–24] and tumor growth,[25–27] antiviral and antibacterial activity,[28–30] and antimutagenic activity.[31] Its effects on enzyme systems include elevation of glutathione *S*-transferase,[26,32] inhibition of cytochrome P-450 reductase,[33] and elevation of lactate dehydrogenase activity.[34,35] Recent studies have shown that garlic affords protection against lipid peroxidation, suggesting it may attenuate the development of atherosclerosis.[36–39] Garlic and other lipophilic antioxidants, such as butylated hydroxytoluene (BHT) and probucol, have also been demonstrated to prevent oxidation of the lipid components of low-density lipoproteins.[40–44]

Although there are numerous reports on antioxidant activities of garlic, a majority of them were carried out using crude compounds or garlic extract.[37,38,44] In the present investigation however, alliin and its analogues, allyl cysteine, allicin, and allyl disulfide, representing the four classes of compounds commonly found in garlic or garlic preparations, were prepared and evaluated for their scavenging activities on superoxide, hydroxyl radical, and hydrogen peroxide, and for their

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ability to prevent microsomal lipid peroxidation (Fig. 1). The pro-oxidant activities of the garlic compounds were also tested by measuring the formation of hydrogen peroxide as some antioxidants such as ascorbic acid have been demonstrated to undergo autooxidation in certain conditions.[45,46]

MATERIALS AND METHODS

Mannitol, ascorbic acid, uric acid, catalase (from bovine liver), superoxide dismutase (from horseradish), xanthine oxidase (grade I, from milk), horseradish peroxidase (HRP) (type I), Chelex-100, and other reagents of the highest purity available were obtained from Sigma Co. Ltd. (Poole, Dorset, UK). Allyl disulfide (technical grade) was from Aldrich Chemical Co. Ltd. (Gillingham, Dorset, UK).

Allyl cysteine and alliin

The procedures adopted were essentially those of Iberl *et al*.[47] Sodium methoxide [30% (wt/wt) in methanol; 18.01 g] was added dropwise to dried L-cysteine (12.14 g; 0.1 mol) precovered with sufficient dry methanol in a dried reaction flask. The exothermic reaction was carried out in a dry nitrogen atmosphere and stirred mechanically until the cysteine crystals had disappeared. Allyl bromide (12.15 g; 0.1 mol) was then added slowly to the reaction mixture, and the reaction was allowed to proceed for 45 minutes until the formation of a white suspension. Sufficient dilute hydrochloric acid was added to neutralize the excess sodium methoxide, methanol was removed from the mixture *in vacuo*, and a minimum quantity of distilled water was added to dissolve the white residue. Silver nitrate (16.99 g; 0.1 mol) dissolved in water was then added to the solution to precipitate the bromide as a silver salt and removed by filtration under reduced pressure. The filtrate was evaporated to dryness *in vacuo*, and the residue was redissolved in an acetone-water mixture to yield crystalline allyl cysteine and then purified by repeated crystallization.

Dried allyl cysteine (2.0 g; 0.012 mol) suspended in glacial acetic acid (50 mL) was cooled and stirred. Hydrogen peroxide [30% (wt/vol); 1.496 g] was added dropwise over 30 minutes, and the reaction proceeded for a further 60 minutes. The unreacted hydrogen peroxide was then destroyed by the addition of acid-washed activated charcoal, followed by the removal of glacial acetic acid in high vacuum, leaving a residue containing both charcoal and crude alliin. The residue was dissolved in a minimum quantity of distilled water and filtered to remove the charcoal. Acetone was then added to the aqueous filtrate and allowed to stand, yielding crystalline alliin. Repeated crystallization was carried out with acetone-distilled water to purify the (+)-alliin. The products were analyzed by melting point, ^{1}H nuclear magnetic resonance (NMR), Fourier transformed-infrared (FT-IR), and thin layer chromatography (TLC).[48] The melting point and infrared spectrum of alliin were consistent with those reported in the literature.[28,47,49]

Allyl cysteine. Analysis gave the following results: yield, 50% (colorless to white crystalline plates); melting point, 217–219°C; ^{1}H NMR (D_2O), δ 3.0 (2H, d, $-$*CH_2-S-C-C · [COOH][NH_2]), 3.2 (2H, d, -C-S-*CH_2-C · [COOH][NH_2]), 3.9 (1H, t, -C-S-C-*CH · [COOH][NH_2]), 5.3 (2H, d, *CH_2=C-C-S-), 5.6–6.1 (1H, m, C=*CH-C-S-); FT-IR (cm^{-1}), 2940 (NH_3^+), 1600 (COO).

(+)-Alliin/(+)-S-allyl-L-cysteine sulfoxide. Analysis gave the following results: yield, 34% (colorless to white crystalline plates); melting point, 164–166°C; ^{1}H NMR (D_2O), δ 3.3 (2H, 2xd, -*CH_2-S-C-C · [COOH][NH_2]), 3.7 (2H, 2x, -C-S-*CH_2-C · [COOH][NH_2]), 4.2 (1H, t, -C-S-C-*CH · [COOH][NH_2]), 5.6 (2H, d, *CH_2=C-C-S-), 5.7–6.1 (1H, m, C=*CH-C-S-); FT-IR (cm^{-1}), 3020 (NH_3^+), 1605 (COO), 1025 (sulfoxide).

Allyl disulfide and allicin

Allyl disulfide (technical grade) was purified by repeated fractional distillation (second fraction) in high vacuum (1 mm Hg). Allicin was prepared by oxidizing purified allyl disulfide with 3-chloroperbenzoic acid according to published methods.[50,51] The products were analyzed by ^{1}H NMR, FT-IR, and TLC, and the spectra were consistent with the literature.[51–53]

Allyl disulfide. Analysis gave the following results: yield, 38% (yellowish-brown oily liquid); boiling point, 190°C; ^{1}H NMR ($CDCl_3$), δ 3.3 (4H, d, [C=C-*CH_2-S]$_2$), 5.1–5.2 (4H, m, [*CH_2 = C-C-S]$_2$), 5.7 (2H, m, [C = *CH-C-S]2); FT-IR (cm^{-1}), 2940 (NH_3^+), 1634 (C=C).

Allicin. Analysis gave the following results: yield, 25% (yellowish-brown oily liquid); ^{1}H NMR ($CDCl_3$), δ 3.7 (4H, m, [C = C-*CH_2-S]$_2$), 5.1–5.6 (4H, m, [*CH_2 = C-C-S]$_2$), 5.8 (2H, m, [C=*CH-C-S]2); FT-IR (cm^{-1}), 3020 (NH_3^+), 1605 (C=C), 1038 (sulfoxide).

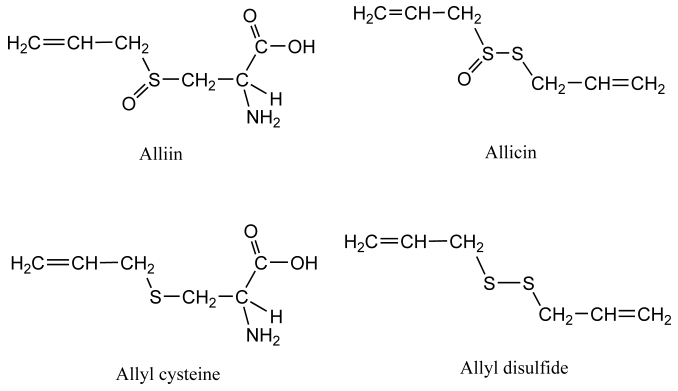

FIG. 1. Chemical structures of alliin, allyl cysteine, allyl disulfide, and allicin.

Superoxide scavenging studies

A spectrophotometric procedure based on superoxide generation by xanthine/xanthine oxidase was adopted.[54,55] The reaction mixture, in a final volume of 3 mL, contained 1 m*M* disodium EDTA, 0.1 m*M* xanthine, test solution, 3 m*M* nitro blue tetrazolium chloride (NBT), and 50 m*M* KH_2PO_4/KOH buffer, pH 7.4. The reaction was initiated by adding 20 μL of xanthine oxidase (1 unit/mL), which gave an absorbance change of 0.020/minute at 560 nm. Solutions of test compounds (alliin, allyl cysteine, allyl disulfide, allicin, uric acid, and ascorbic acid) were freshly prepared, and the pH was adjusted to 7.4 where necessary. Controls without addition of xanthine oxidase were run in parallel to assess the interaction of the test compounds with NBT. To determine the inhibitory effects of test compounds on superoxide generation system, the formation of uric acid in the reaction mixture without the addition of NBT was measured spectrophotometrically at 295 nm.

Hydroxyl scavenging studies

The procedure described by Halliwell *et al*.[56] was adopted with minor modifications. Reaction mixtures, in a final volume of 1.0 mL, consisted of the following reagents: 20 m*M* KH_2PO_4/KOH buffer (pH 7.4), 2.8 m*M* deoxyribose, 10 μM iron (III) chloride ($FeCl_3$)/100 μM EDTA, 1 m*M* hydrogen peroxide, 100 μM ascorbic acid, and test compounds (alliin, allyl cysteine, allyl disulfide, allicin, mannitol, and ethanol; 0–8 m*M*). Solutions of $FeCl_3$ and ascorbic acid were freshly prepared with deaerated water before use. The reaction mixtures were incubated at 25°C for 1 hour. The pink chromogen from the oxidatively damaged deoxyribose was developed by adding 1 mL of 1% (wt/vol) thiobarbituric acid (TBA) in 0.05 M NaOH and 1 mL of 2.8% (wt/vol) trichloroacetic acid (TCA), and the mixture was heated at 100°C for 30 minutes. The absorbance of the pink adduct of malondialdehyde (MDA) and TBA (MDA-TBA) was determined spectrophotometrically at 532 nm.

To validate hydroxyl scavenging activity, the potential interaction of hydrogen peroxide and the test compounds, the inhibitory effect of the test compounds on the formation of MDA-TBA derivatives from deoxyribose degradation products and TBA, and the reactivity of the test material with TBA to form MDA-TBA derivatives were assessed, and the methods used were as outlined below.

Interaction with hydrogen peroxide. Reaction mixtures, in a final volume of 1.0 mL, consisted of the following reagents: 20 m*M* KH_2PO_4/KOH buffer (pH 7.4), 1 m*M* hydrogen peroxide, and test material (alliin, allyl cysteine, and allyl disulfide; 0–8 m*M*). Solutions of hydrogen peroxide and the materials were made up freshly with Chelex-100-treated redistilled water. The reaction mixtures were incubated at 25°C for 1 hour as in the hydroxyl scavenging studies. This was followed by the addition of 9 mL of redistilled water and thorough mixing. Twenty-microliter aliquots of the reaction mixtures were immediately withdrawn and assayed for hydrogen peroxide using the scopoletin-HRP procedure. The hydrogen peroxide assay solution consisted of 50 m*M* KH_2PO_4/KOH buffer (pH 7.4), 0.5 μM scopoletin, and 0.8 unit/mL HRP in a final volume of 3.0 mL. The reaction was initiated by the addition of HRP. The change in fluorescence (emission, 320 nm; excitation, 460 nm) of the reaction mixture with or without prior addition of catalase represented the hydrogen peroxide content in the test solution, which was derived from a standard hydrogen peroxide concentration curve.

Effects of alliin, allyl cysteine, allicin, and allyl disulfide on formation of MDA-TBA products. Reaction mixtures, in a final volume of 1.0 mL, consisted of the following reagents: 20 m*M* KH_2PO_4/KOH buffer (pH 7.4), 2.8 m*M* deoxyribose, 10 μM $FeCl_3$/100 μM EDTA, 1 m*M* hydrogen peroxide, and 100 μM ascorbic acid. The reaction mixtures were incubated at 25°C for 1 hour; then 1% (wt/vol) TBA (1 mL) in 0.05 *M* NaOH, 5.6% (wt/vol) TCA (0.5 mL), test compound (alliin, allyl cysteine, allicin, and allyl disulfide; up to 8 m*M*), and redistilled water were added to the reaction mixtures to give a final volume of 3.0 mL. The mixture was heated at 100°C for 30 minutes, and the absorbance was was determined spectrophotometrically at 532 nm.

Reactivity of alliin, allyl cysteine, allicin, and allyl disulfide with TBA to form MDA-TBA products. Reaction mixtures, in a final volume of 1.0 mL, consisted of the following reagents: 20 m*M* KH_2PO_4/KOH buffer (pH 7.4), with and without the presence of 2.8 m*M* deoxyribose and test compound (alliin, allyl cysteine, allicin, and allyl disulfide). The reaction mixtures were incubated at 25°C for 1 hour; then 1% (wt/vol) TBA (1 mL) in 0.05 *M* NaOH and 5.6% (wt/vol) TCA (1 mL) were added to the reaction mixtures to give a final volume of 3.0 mL. The mixture was heated at 100°C for 30 minutes, and the absorbance was measured at 532 nm.

Lipid peroxidation studies

Liver microsomes were prepared from male Wistar rats weighing 200–250 g as previously described.[57] The washed microsomal pellet obtained was suspended in 0.1 *M* sodium phosphate buffer, pH 7.4. Protein concentration of the microsomal preparation was determined using a modified Lowry method, with bovine serum albumin as the standard.[58]

The lipid peroxidation study was based on the procedure described by Itoh *et al*.[59] Microsomes (1.5 mg of protein/mL) were preincubated with test compound (0.1–6.0 m*M*) at 37°C for 5 minutes. This was followed by addition of 0.1 m*M* ascorbic acid and 5 μM ferrous sulfate to initiate lipid peroxidation, and the reaction mixtures (1 mL, final volume) were incubated at 37°C for 3 hours. The reaction was terminated by the addition of 1 m*M* EDTA. To

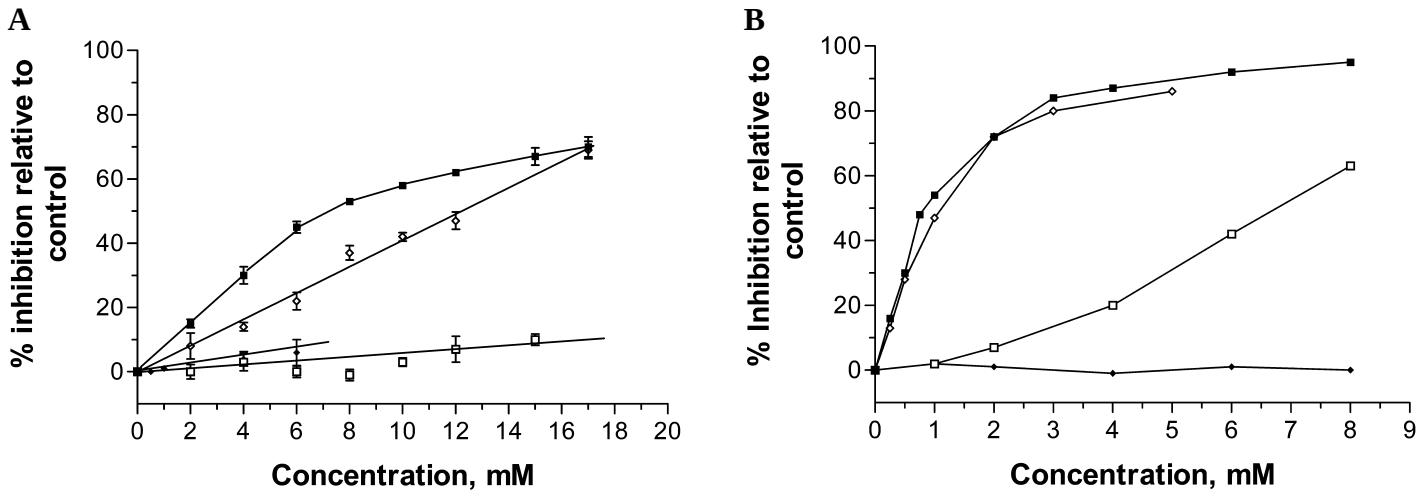

FIG. 2. (**A**) Superoxide scavenging (% inhibition of NBT reduction) by alliin (■), allyl cysteine (■), allyl disulfide (◆), and allicin (◇) at 25°C. (**B**) Superoxide scavenging (% inhibition of NBT reduction) by uric acid (■), glutathione (□), mannitol (◆), and ascorbic acid (concentration, $\times 10^{-2}$ m*M*) (◇) at 25°C. Each data point is expressed as mean ± SD($n = 5$).

assay for MDA formed during lipid peroxidation, the procedure based on TBA described previously was adopted.[60] The absorbance of the reaction mixture containing MDA-TBA adducts was measured at 535 nm.

Hydrogen peroxide formation

A procedure based on the decrease in fluorescence of scopoletin in the presence of HRP and hydrogen peroxide reported previously was adopted.[61] The reaction mixture contained, in a final volume of 6 mL, 5×10^{-7} *M* scopoletin, test material (alliin and allyl cysteine; 2.5–10 m*M*), and 50 m*M* KH_2PO_4/KOH buffer, pH 7.4. The reaction was started by adding 60 μL of HRP (80 units/mL). The reaction was agitated and kept in the dark at 37°C. The change in fluorescence of the mixture was monitored at time intervals (excitation, 320 nm; emission, 460 nm). The catalase control consisted of 5×10^{-7} *M* scopoletin, test material (2.5–10 m*M*), 400 units/mL catalase, 0.8 units/mL of HRP, and 50 m*M* KH_2PO_4/KOH buffer (pH 7.4) in a final volume of 6 mL. The scopoletin control consisted of 5×10^{-7} *M* scopoletin, test material (2.5–10 mM), and 50 m*M* KH_2PO_4/KOH buffer, pH 7.4.

The level of hydrogen peroxide formed is indicated by the difference in the fluorescence of the reaction mixtures containing test material with and without catalase. The level of hydrogen peroxide generated by the test material, expressed in *M*, was derived from a standard concentration curve constructed with known concentrations of standard hydrogen peroxide (10^{-6}–10^{-8} *M*).

RESULTS

Superoxide scavenging

The results depicted in Figure 2A show the superoxide scavenging activity of alliin, allyl cysteine, and allyl disulfide based on NBT reduction assays at 25°C. The superoxide scavenging activity of alliin was significantly greater than that of allyl cysteine and allyl disulfide (one-way analysis of variance; Tukey's *post hoc* comparisons, $P < .05$). The results suggested alliin scavenged superoxide efficiently but its analogues, allyl cysteine and allyl disulfide, were relatively inactive. Allyl disulfide was not evaluated above 6 m*M* because of its low solubility in the aqueous assay system. The low aqueous solubility may contribute to its inability to scavenge superoxide. Allicin appears to be a moderately active superoxide scavenger (Fig. 2A); however, the rates of NBT reduction and the concomitant inhibition of uric acid formation (superoxide) were similar (Figs. 2A and 3). The reduction in uric acid formation was probably me-

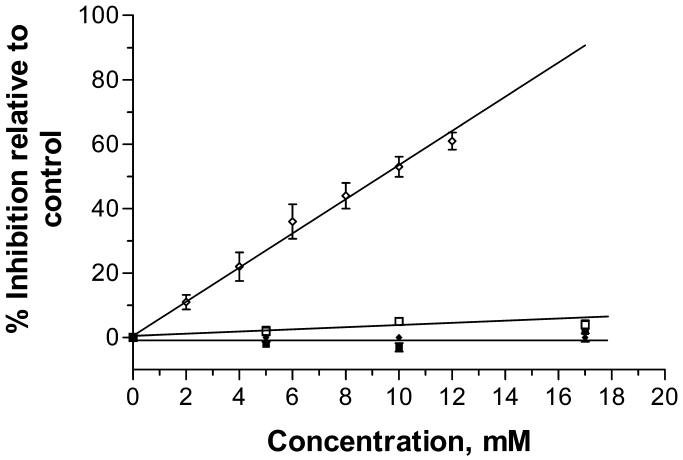

FIG. 3. Inhibition of uric acid formation by the xanthine/xanthine oxidase system in the presence of alliin (■), allyl cysteine (□), allyl disulfide (◆), and allicin (◇) at 25°C. Each data point is expressed as mean ± SD ($n = 5$).

TABLE 1. COMPARISON OF RATE CONSTANTS FOR HYDROXYL SCAVENGING ACTIVITY OF ALLIIN, ALLYL CYSTEINE, ALLYL DISULFIDE, AND OTHER SCAVENGERS AS DETERMINED BY DEOXYRIBOSE/TBA ASSAY

	Rate constant (M^{-1} s^{-1})	*Reference*
Alliin	13.8–16.6 × 10^9	Present study[a]
	41.0–6.0 × 10^9	Kourounakis and Rekka[37,b]
Allyl cysteine	2.1–2.2 × 10^9	Present study[a]
Allyl disulfide	0.7–1.5 × 10^{10}	Present study[a]
Allicin	NA	Present study[a]
Mannitol	1.0–2.0 × 10^9	Present study[a]
	1.3–1.4 × 10^9	Halliwell *et al.*[56]
Ethanol	1.0–1.5 × 10^9	Present study[a]
	1.2–1.4 × 10^9	Halliwell *et al.*[56]
Salicyclic acid	6.0–10.0 × 10^9	Aruoma and Halliwell[63]
Indomethacin	9.8–12.0 × 10^9	Aruoma and Halliwell[63]
Ketoprofen	7.3–10.0 × 10^9	Aruoma and Halliwell[63]

[a]n = 5 experiments. NA, no hydroxyl scavenging activity was detected.
[b]The procedure was based on the competitive inhibition by the test compound on the degradation of dimethyl sulfoxide by hydroxyl.

diated by the inhibitory effect of allicin on xanthine oxidase, which suppressed the production of superoxide. Hence, the dose–response NBT reduction is not a result of superoxide scavenging activity. Allicin has been shown to inhibit enzymes by affecting sulfhydryl groups,[62] and this may explain the inhibitory effect of allicin on the enzyme system to form superoxide. The 50% (median) inhibitory concentration (IC_{50}) of these compounds indicates that alliin (IC_{50} = 7.1 m*M*) is significantly less efficient than uric acid (IC_{50} = 0.67 m*M*) and ascorbic acid (IC_{50} = 0.01 m*M*) as a superoxide scavenger while being only slightly less potent than glutathione (IC_{50} = 6.8 m*M*). The result also indicates that mannitol does not react with superoxide (Fig. 2B).

To eliminate the possibility of false-positive results, we have examined the effects of alliin, allyl cysteine, allicin, and allyl disulfide on the superoxide generation system by monitoring the formation of uric acid. Apart from allicin, other test compounds did not interfere with uric acid formation, and hence there was no inhibition of xanthine oxidase (Fig. 3). This suggested that the generation of superoxide was unaffected by alliin, allyl cysteine, and allyl disulfide, and the possibility of false-positive results was ruled out.

Hydroxyl scavenging

The deoxyribose assay usually gives similar results to the definitive technique of pulse radiolysis.[56] The accuracy and reproducibility of the deoxyribose method adopted are confirmed as the rate constants of ethanol and mannitol obtained here are consistent with the literature (Table 1).

The hydroxyl scavenging activity assays demonstrated that alliin, allyl cysteine, and allyl disulfide show high activity, while allicin appeared not to react with the hydroxyl radical efficiently (0.1–0.5 m*M* and 0.5–8.0 m*M*, respectively) (Figs. 4 and 5). This is unlikely to be a false-positive since in the control studies, alliin, allyl cysteine, and allyl disulfide did not react with hydrogen peroxide or affect the formation of MDA-TBA derivatives from hydroxyl damaged deoxyribose and TBA (data not shown). The deoxyribose competition plot of allyl cysteine with hydroxyl is linear and hence obeys second-order kinetics. In contrast, the competition plot for alliin is non-linear at high concentrations (0.5–8.0 m*M*) with EDTA/$FeCl_3$/hydrogen peroxide as the hydroxyl generation system (Fig. 4). At low concentrations, both alliin and allyl disulfide followed second-order kinetics (0.1–0.5 m*M*) (Fig. 5). The rate constant of the plots suggests alliin and allyl disulfide are similar in reactivity towards hydroxyl radicals and that both are more efficient than allyl cysteine and other scavengers such as mannitol, ethanol, and some anti-inflammatory drugs (Table 1 and Fig. 4). The reaction kinetics for alliin obtained in this experiment are about 2.5 times lower than

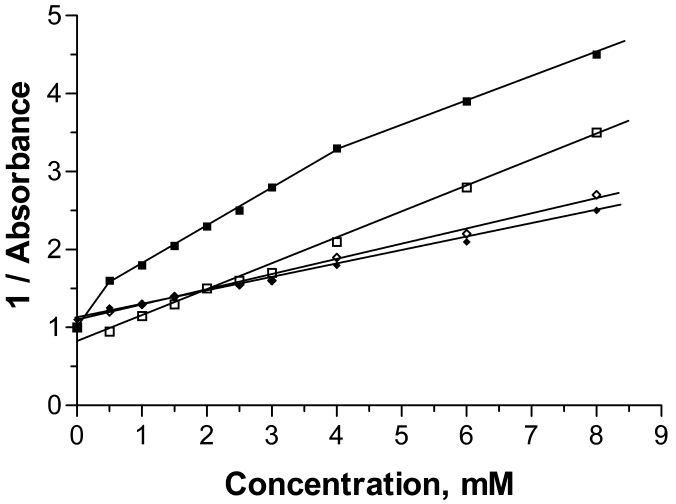

FIG. 4. Hydroxyl scavenging by alliin (■), allyl cysteine (□), mannitol (◆), and ethanol (0.5–8.0 m*M*) (◇) at 25°C. Each data point is expressed as mean ± SD (n = 5).

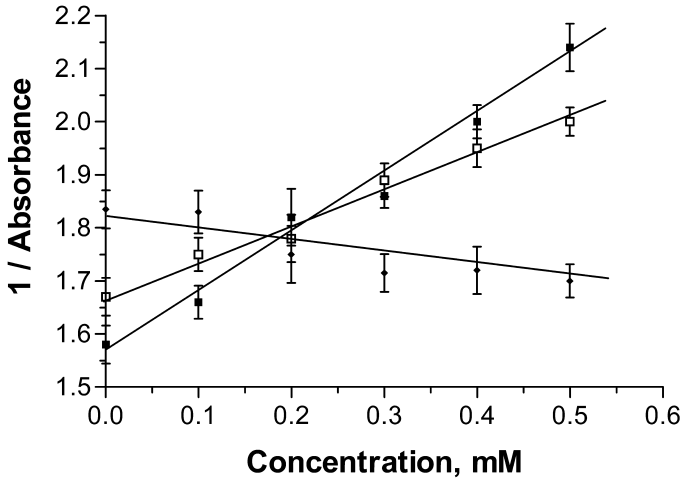

FIG. 5. Hydroxyl scavenging by alliin (■), allyl disulfide (□), and allicin (0.1–0.5 m*M*) (◆) at 25°C. Each data point is expressed as mean ± SD ($n = 5$).

that reported by Kourounakis and Rekka[37] (Table 1). The discrepancy may have arisen as a result of the different procedures adopted.

The non-linear competition plots for alliin suggest that there is some interaction between alliin and one or more of the components of the reaction mixture. It has previously been shown that the ability of a scavenger to react with hydrogen peroxide or chelate iron, for example, may lead to a non-linear plot.[56] Hence, the likely explanation for the anomalous results is the ability of alliin to chelate iron and hence to cause a redistribution of iron between complexants present in the reaction mixture.

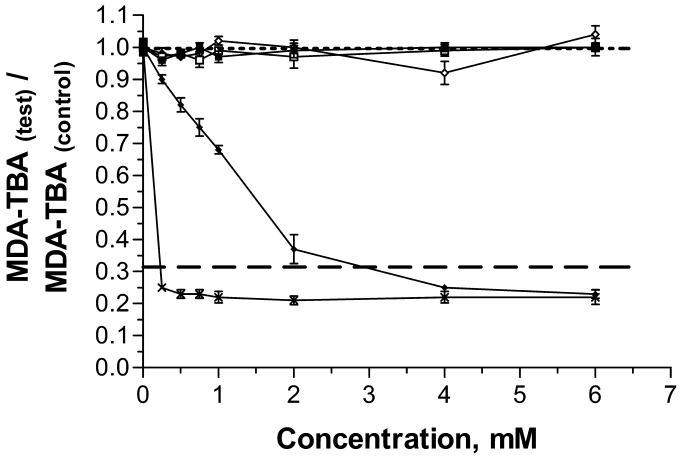

FIG. 6. Prevention of microsomal lipid peroxidation by alliin (■), allyl cysteine (□), allyl disulfide (◆), allicin (◇), and BHT (×) at 37°C. Control assays included microsomes + ferrous sulfate + ascorbic acid (– – –) and microsomes only (— —). Each data point is expressed as mean ± SD ($n = 5$).

Microsomal lipid peroxidation

The formation of MDA-TBA indicates that the occurrence of peroxidation in membranes, and the results depicted in Figure 6 show allyl disulfide protected microsomal membranes from ferrous sulfate/ascorbic acid-induced lipid peroxidation, at concentrations above about 2.0 m*M*, and its formation of MDA-TBA was significantly different from those induced by alliin, allicin, and allyl cysteine and by the control (microsomes + ferrous sulfate + ascorbic acid) (one-way analysis of variance; Tukey's *post hoc* comparisons, $P < .05$). Alliin, allicin, and allyl cysteine did not appear to reduce lipid peroxidation under the same conditions, and their formations of MDA-TBA were similar to that by the control (microsomes + ferrous sulfate + ascorbic acid) (one-way analysis of variance; Tukey's *post hoc* comparisons, $P > .05$). The positive control (BHT) protected the membranes from peroxidation at all concentrations tested. This indicates that allyl disulfide is an effective inhibitor of lipid peroxidation, although it is less potent than BHT.

Hydrogen peroxide formation

Alliin shows dose-related increases in hydrogen peroxide formation until reaching concentrations of 5 m*M*, whereas allyl cysteine did not generate any hydrogen peroxide under the same conditions (Fig. 7). Allyl disulfide and allicin were not evaluated as they have low solubility at high concentrations. Alliin generated low levels of hydrogen peroxide, about 0.5 (10^{-77} *M*/hour (2.5 m*M* alliin) and 1×10^{-7} *M*/hour (5 m*M* alliin) (about 1 molecule of hydrogen peroxide was formed per 50,000 molecules of alliin per hour for 2.5 and 5.0 m*M* alliin). At 10 m*M* alliin, the level of hydrogen peroxide formed was similar to that by 5 m*M* alliin (Fig. 7) (one-way analysis of variance; Tukey's *post hoc* comparisons, $P > .05$). Hence, the pro-oxidant activity of alliin is likely to be insignificant, and this is supported by the results of the lipid peroxidation studies.

Since the molecular structure of alliin suggests it does not generate hydrogen peroxide directly, it is likely that the hydrogen peroxide was formed by the spontaneous dismutation of superoxide, which in turn arose from the autoxidation of alliin in the presence of oxygen. Such autooxidation will have involved catalysis by a suitably complexed transition metal ion such as $Fe^{2+/3+}$ or $Cu^{+/2+}$. The results of the hydroxyl scavenging studies suggest that alliin complexes with iron. In contrast, allyl cysteine appeared to be incapable of undergoing such complexation reaction. This probably explains the difference of alliin and allyl cysteine in terms of hydrogen peroxide formation.

DISCUSSION

The efficacy of garlic preparations or extracts to alleviate free radical damage to biological membranes or other biological systems has been demonstrated.[31,37,38] However, evidence on the components responsible for the activity is rather limited. This study clearly showed that the garlic com-

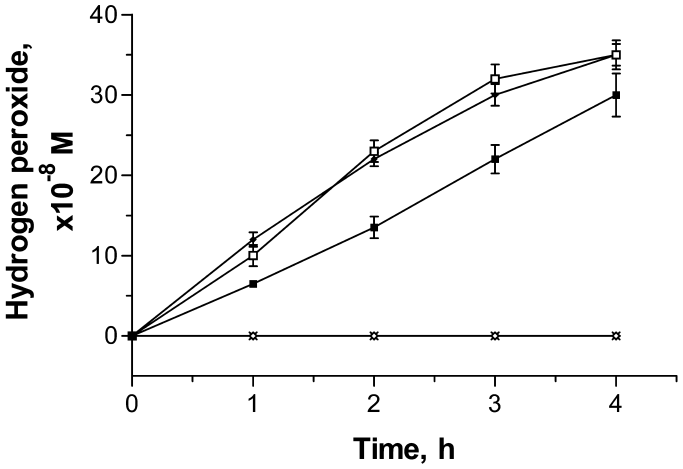

FIG. 7. Formation of hydrogen peroxide by alliin and allyl cysteine in 50 m*M* KH_2PO_4-KOH (pH 7.4) at 37°C: 2.5 m*M* alliin (■), 5 m*M* alliin (□), 10 m*M* alliin (◆), 2.5 m*M* allyl cysteine (◇), 5 m*M* allyl cysteine (×), and 10 m*M* allyl cysteine (+). No hydrogen peroxide formation was detected for the three concentrations of allyl cysteine. Each data point is expressed as mean ± SD ($n = 5$).

pounds tested have different patterns of antioxidant activity in terms of scavenging of superoxide and hydroxyl radical, and preventing peroxidation of microsomal membranes. For example, alliin scavenges superoxide as generated by the xanthine/xanthine oxidase system, while allyl cysteine, allicin, and allyl disulfide did not react with superoxide.

It has been suggested by Kourounakis and Rekka[37] that the hydroxyl scavenging activity of a garlic extract was probably a result of the presence of allicin, which originated from alliin by the catalytic action of alliinase. Prasad *et al.*[64] indicated that Garlicin (Nature's Way, Springville, UT), a commercial garlic preparation containing alliin, scavenged hydroxyl radical using salicylic acid as the detector molecule. More recently, Rabinkov *et al.*[62] also reported that allicin generated from alliin by allinase inhibits the scavenging of hydroxyl and methyl radicals by the spin trap, 5,5′-dimethyl-1-pyrroline *N*-oxide.

In contrast, this study has clearly shown that purified allicin did not scavenge hydroxyl radicals in the deoxyribose competitive assay or in the *in vitro* lipid peroxidation study. The reason for the discrepancy has not been ascertained. However, it is conceivable the relative reactivity of the detector molecules, deoxyribose, 5,5′-dimethyl-1-pyrroline *N*-oxide, and salicylic acid towards hydroxyl radical may explain the differences. Another reason could be the presence of trace contaminants such as allyl disulfide and alliin in previous studies since allicin was prepared by enzymic action of allinase on alliin or allicin present in the commercial preparation, Garlicin.[37,62,64]

The rate constants of alliin, allyl cysteine, and allyl disulfide reactions with hydroxyl radical, according to the deoxyribose competitive assay, is high ($1.4–1.7 \times 10^{10}$, $2.1–2.2 \times 10^{9}$, and $0.7–1.5 \times 10^{10}$ M^{-1} second^{-1}, respectively, which indicates that they are efficient scavengers when compared with other antioxidants such as mannitol. In contrast, alliin, allyl cysteine, and allicin were ineffective in preventing lipid peroxidation of microsomal membranes, while allyl disulfide protected the microsomal membranes from induced lipid peroxidation, at concentrations above about 2.0 m*M*. This could be explained by the partition coefficients of the compounds; alliin [log (*P*), −1.93] and allyl cysteine [log (*P*), 0.03] are highly water soluble and remain in the aqueous pool, while allyl disulfide [log (*P*), 2.95] is lipophilic and hence associates with the lipid phase or biological membranes. This probably enables allyl disulfide in the lipid phase to scavenge oxidants generated near the proximity of biological membranes more efficiently than alliin and allyl cysteine. This is consistent with the literature, which most of the antioxidants, *e.g.*, tocopherols, BHT [log (*P*), 5.64], *N*,*N*′-diphenyl-*p*-phenylene diamine [log (*P*), 4.96] [estimated using ChemDraw Ultra version 6.0 (CambridgeSoft, Cambridge, MA)], etc., used in lipid peroxidation studies are fairly lipophilic.[2,3]

Paradoxically, some highly efficient antioxidants autooxidize in certain conditions, generating highly reactive oxygen species, which damage proteins and biological membranes. The lipid peroxidation and hydrogen peroxide formation studies suggest they are unlikely to behave as potent pro-oxidants in both aqueous and lipid phases.

In summary, the aqueous and lipid-soluble garlic compounds such as alliin, allyl disulfide, and allyl cysteine possess different patterns of antioxidant activities as shown in Table 2. We have clearly shown that allicin suppresses the enzymic activity of xanthine oxidase to generate superoxide. It also lacks hydroxyl radical scavenging activity and

TABLE 2. SUMMARY OF ANTIOXIDANT ACTIVITIES AND PARTITION COEFFICIENTS [LOG(*P*)] OF ALLIIN, ALLICIN, ALLYL CYSTEINE, ALLYL DISULFIDE

Compound	*log*(P)[a]	*Superoxide scavenging*[b]	*Hydroxyl scavenging*[b]	*Lipid peroxidation*[b]
Alliin	−1.93	+	+	−
Allicin	1.35	I	−	−
Allyl cysteine	0.03	−	+	−
Allyl disulfide	2.95	−	+	+

[a]Partition coefficients in *n*-octanol/water were estimated using ChemDraw Ultra version 6.0 (CambridgeSoft).
[b]+, activity detected; −, no activity found; I, inhibits xanthine oxidase.

does not suppress lipid peroxidation. Therefore, it is conceivable that the reported antioxidant activity of allicin is probably mediated *via* its ability to inhibit enzymes that promote pro-oxidant status via thiol exchange. Therefore, biologically these garlic compounds may contribute to the endogenous antioxidant pool of compounds such as ascorbic acid, tocopherol, uric acid, glutathione, and other thiols[12] and to enzyme suppression. The overall effect of the garlic compounds would perhaps be to prevent or reduce injuries through oxidative stress and free radicals.

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