Redox-Sensitive Proteins Are Potential Targets of Garlic-Derived Mercaptocysteine Derivatives¹⁻³

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ABSTRACT Molecular investigations support existing clinical and epidemiological data that garlic-derived allylsulfides reduce cancer risk. Various allylsulfides can diminish progression of cancer cells at either the G₂/S or G₂/M phase. Allylsulfide derivatives modify redox-sensitive signal pathways and cause growth inhibition, mitotic arrest, and apoptosis induction. Whether allylsulfides modify intracellular redox potentials by affecting the ratio of glutathione:glutathione disulfide and/or by interacting directly with sulfhydryl domains on regulatory or catalytic-signal proteins requires further investigation. To understand the possible biochemical mechanisms contributing to the protective effects of allylsulfides, we investigated the ability of these compounds to undergo enzyme-catalyzed transformations. In addition to catalyzing γ-elimination reactions, γ-cystathionase can perform β-elimination reactions with cysteiny1 S-conjugates derived from garlic extracts when the S-alkyl group (R) is larger than ethyl. The reaction products are pyruvate, ammonium, and a sulfur-containing fragment (RSH). β-Lyase substrates of γ-cystathionase thus far identified from garlic include: S-allyl-L-cysteine (R = CH₃-CHCH₂-), S-allylmethio-L-cysteine (R = CH₃-CHCH₂S-), and S-propylmercapto-L-cysteine (R = CH₃CH₂CH₂S-). Mercapto derivatives yield persulfide products (RSSH) that are potential sources of sulfane sulfur, which may modify protein function by reacting at important cysteinyl domains. Thus, β-elimination reactions with cysteine S-conjugates in garlic may modify cancer-cell growth by targeting redox-sensitive signal proteins at sulfhydryl sites, thereby regulating cell proliferation and/or apoptotic responses. These interactions may be useful in identifying efficacy of garlic-derived compounds and/or developing other novel organosulfur compounds that may modify intracellular redox potentials or interact with thiols associated within cysteine domains in regulatory, catalytic, signal, or structural proteins. J. Nutr. 136: 835S–841S, 2006.

KEY WORDS: • garlic • β-lyase; glutathione • cysteine S-conjugates • γ-cystathionase • sulfane sulfur

Epidemiologic investigations have shown that the risk of several types of cancers is inversely related to intake of garlic (Allium sativum) (1,2). Meta-analyses of this literature revealed that increased garlic consumption diminished the risk of stomach and colorectal cancers, but efficacy against developing other types of cancers was either equivocal or required additional study (3). In one case-control study of prostate cancer (4), risk was significantly less in individuals who regularly consumed garlic food items 2 or more times per week. There was a significant trend of decreasing risk with an increase in garlic consumption of less than once per month, increasing to 1–4 times per mo, and then to 2 or more times per week (P = 0.038). In a subsequent population-based, case-control study, risk of prostate cancer was decreased with intake of allium vegetables (5). This risk was independent of body size, intake of other foods, and total calorie intake, and was more pronounced for men with localized rather than advanced prostate cancer.
These findings provide incentives for further mechanistic evaluations of the potential effects of garlic constituents to control growth and apoptosis of various organ-site cancers.

**Relevance of garlic extracts to carcinogenesis and cell proliferation.** Organosulfur compounds isolated from garlic display antitumor potential in a number of carcinogen model systems (reviewed in 6). The biochemical mechanisms that underlie the antimutagenic, antitumorigenic, and antiproliferative effects of garlic-derived organosulfur compounds are not precisely known. Several animal model studies demonstrate that allylsulfides, in particular diallylsulfide, diallylthiolsulfide, allyl methylsulfide, and ajoen (CH$_2$=CH=CH-S(O)CH$_2$CH=CH-S-S-CH$_2$CH=CH$_2$) specifically modulate the activities of cytochrome P450 (Cyp P450) mixed-function oxidases and thus inhibit tumors induced by a wide variety of carcinogens (nitrosamines, benz[a]pyrene, dimethylbenz[a]anthracene, azoxymethane) bioactivated by Cyp P450s (7,8). The effect of allylsulfides on modifying Cyp P450s appears to be specific rather than general and is supported by the observation that the activities of several Cyp P450s (e.g., Cyp 1A1, 1A2, 3A4, 2B1, 2B4) are increased by various allyl organosulfur components, and others are downregulated (9–11). Concurrent with selective modification of phase I P450 enzymes, organosulfides facilitate detoxification of carcinogens through upregulation of phase II-conjugating enzymes such as glutathione S-transferases, epoxide hydrolase, quinone reductase, and UDP-glucuronosyltransferase (12–15). In addition, induction of glutathione peroxidase and an increase in the ratio of glutathione (GSH) to glutathione disulfide (GSSG) may contribute to the protective effects of organosulfur compounds in diminishing oxidative stress within cells (16,17). Collectively, these cellular responses to organosulfides can partially account for the cancer-preventing effects of garlic-derived allylsulfides in various target organs.

In addition to blocking chemical carcinogenesis, S-allylsulfides inhibit growth of a variety of transplantable tumors and exert antipromotional activity against hormone-responsive (prostate, mammary) and refractory (gastric, colon) mammalian tumor cell lines (6,18–25). Accordingly, a variety of mono-, di-, and tri-organosulfide derivatives bearing an S-allyl moiety can directly or indirectly up- and downregulate a number of cell-cycle checkpoint control proteins (26–28), apoptotic regulatory proteins (20,29–31), as well as signal transduction and transcription factors (22,32–36).

A change in the redox environment of these proteins, or in the extent of phosphorylation or dephosphorylation of these proteins by redox-sensitive kinases or phosphatases, are potential mechanisms by which organosulfur compounds can attenuate or enhance the response of cell-signaling pathways. In some cases, increased phosphorylation of these proteins correlates with the antiproliferative activity of organosulfur compounds on normal smooth-muscle and endothelial cells (25,37). Flow cytometric analyses of DNA indicate that some S-allylsulfides can prevent some tumor-cell lines from progressing through the G$_1$/S phase while other tumor-cell lines are blocked in G$_2$/M (25,33,38). Thus, S-allylsulfides may potentially delay or block expression of malignancy or that demonstrate antiproliferative activity on tumor cells by modifying signal transduction mechanisms (40). This last category has attracted recent scientific attention and has been supported by numerous proteomic studies and appears to be most feasible in controlling cellular metabolism.

**Mechanisms of garlic anticancer activity.** Based on experimental data from animal and cell-culture studies, a number of mechanism-based hypotheses suggest how garlic extracts can prevent cancer (see reviews 6,40). These include findings that S-allylsulfides: 1) act as key enhancers of endogenous levels of GSH and glutathione peroxidases, which protect against oxidative damage to DNA, proteins, and membrane lipids; 2) prevent and/or detoxify intermediate metabolites of chemical carcinogens; 3) stimulate immune responses; 4) are metabolized to intermediate metabolites that modify cell cycle and apoptotic factors; and 5) modulate thio-redox reductase, protein disulfide isomerase, quinone reductase, glutathione reductase, and intercellular redox status, which, in turn, regulate cell-signal transduction, transcription factor activation, and DNA repair. Among the potential chemopreventive mechanisms of organosulfur compounds, cell-cycle regulation and apoptotic signaling have received considerable attention. Moreover, they have been extensively studied in vitro and appear to be fundamental to the anticancer effects of garlic. Although these effects are dramatic and reproducible in cell-culture models, the molecular mechanisms need to be elucidated and confirmed in animal studies, and then translated to clinical investigations.

Cell-redox modulation has recently received increased attention because S-cysteinyl organosulfur derivatives (particularly those bearing the more reactive “allyl” rather than a “propyl” moiety) can alter the intracellular redox state that, in turn, activates a number of transcription factors, cell-cycle regulatory proteins, and signal-transduction molecules (41,42).

Induction of cell-cycle arrest or apoptosis is the most common finding in studies in which cells in culture are treated with S-allylsulfides at concentrations that could be attained through dietary consumption. A number of proteins that regulate cellular proliferation can be altered by S-allylsulfides and are considered to be the target proteins involved in the anticancer activity of garlic. A variety of studies show that expression of protein kinase C, cyclin-dependent kinase 2, AP-1, NF-kB, p53, p21$^{waf1}$, and p27$^{kip1}$ are changed most consistently by garlic treatment, especially those of p53 and NF-kB (43–46).

Generally, genetic expression, protein levels, and activities of cell-growth stimulatory proteins are decreased, while those of cell-growth inhibitory proteins are increased by organosulfide derivatives. Cell-cycle arrest has been observed at both G1/S and G2/M cell-cycle checkpoints (47,48). Although induction of apoptosis by garlic-derived S-allylsulfides is well described, specific pathways and important interactions among signal proteins are not well characterized. Experimental data suggest that oxidative stress may play a role in this process and that mitochondria may be the main target for triggering apoptosis based on the observation of cytochrome c release into the cytosol and the activation of caspases following organosulfide treatment. For example, Xiao et al. (20,33) identified interactions among key cellular events that lead to apoptosis following treatment with S-allylmercaptocysteine (SAMC) in colon-cancer cells. Soon after cellular entry, i.e., within 10 min,
SAMS interacts directly with tubulin and initiates microtubular depolymerization. Shortly thereafter (~15 min), SAMS induces the c-Jun NH(2)-terminal kinase pathway that leads directly to activation of caspase-3, poly(ADP-ribose) polymerase, and to other events that mediate an early phase of apoptosis. Concurrent with these events, SAMS also interferes with assembly of mitotic spindles, thus arresting cells in mitosis within the first 12–24 h. Mitotic arrest of cells undoubtedly triggers spindle-checkpoint mechanisms that culminate in late-phase apoptosis. In addition, other seemingly independent pathways become activated by SAMS, such as ERK1/2 and p38 MAP kinase. Thus, these molecular events occur ~24–48 h after exposure of cells to SAMS.

**Biological effects of reactive oxygen species.** Accumulating evidence indicates that intracellular redox status and redox-mediated regulation of signal transduction and gene expression are fundamental mechanisms in cell biology, and that reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) are key players in this process (49–51). ROS generally cause oxidative stress, which may result in a variety of pathologic states, including carcinogenesis and cell apoptosis. In addition, ROS regulate gene expression by direct participation in cell signaling (52) and/or modulate intracellular redox state (53).

Redox changes alter intracellular GSH levels and affect the integrity of sulfhydryl groups in numerous regulatory proteins that control cell growth and differentiation (54–63). Common targets for ROS in these proteins are highly conserved cysteine domains whose redox status and molecular environment are crucial for activity, especially if the modulation occurs at DNA-binding sites (64). Thus, various cellular functions such as gene expression, signal transduction, and enzyme activity can be effectively controlled by the intracellular redox potential principally governed by the ratio of GSH to GSSG (64). Oxidative damage of these sulfhydryl centers may be critically important to the function of signal-transduction and transcription events that utilize redox-sensitive proteins containing these reactive sites.

For example, when cells are treated with diethylmaleate, an agent that depletes endogenous GSH, mRNA levels for these proteins increase. Conversely, when cells are pretreated with sodium metabisulfite (e.g., N-acetylcysteine, diithiothreitol), which inhibits signal transduction presumably by reducing disulfide bridges, induction of specific mRNA for these proteins by diethylmaleate is inhibited, suggesting that expression is stimulated by ROS or RNS accumulation (65). Emerging laboratory data also suggest that exposure of cancer cells to allylsulfides, cysteinyl S-conjugates, or other antioxidant phytochemicals affects biochemical changes in redox-mediated regulation of signal transduction and gene expression (65).

**Post-translational modification of proteins by S-glutathiolation.** As stated earlier, protein thiols (cysteine moieties) are particularly susceptible to oxidative changes within cells and thus can be modified by reversible cysteine-targeted oxidation to regulate enzymic function or alter protein structure (64,65). Post-translational modification of protein sulfhydryl moieties can be achieved by a variety of mechanisms that involve formation of S-cysteinyl mixed disulfides with vicinal cysteiny1 moieties located either on the same protein or on neighboring proteins. Such interactions, depending on the intrinsic nature of the protein or enzyme, may either stabilize or inactivate it. In addition to protein–protein interactions, mixed disulfides are produced with a variety of endogenously and exogenously derived S-cysteinyl compounds. One recognized mechanism that has received considerable attention involves the covalent attachment of the antioxidant tripeptide, glutathione, in either its oxidized (GSSG) or reduced (GSH) form, to proteins via a thiol or disulfide exchange mechanism (64). Numerous studies show that consumption of garlic, as well as cysteinyl S-conjugates, enhance endogenous production of GSH.

GSH is the major intracellular redox buffer that can be oxidized to GSSG, either enzymatically through glutathione peroxidase, or nonenzymatically via ROS or RNS, or that can form mixed disulfides with, for example, S-mercaptopropionyl compounds isolated from garlic. When oxidative stress increases the intracellular ratio of GSSG:GSH, GSSG can undergo thiol-disulfide exchange with cysteinyl residues in proteins resulting in the formation of a protein-mixed disulfide (P-SSG) (Fig. 1A). Alternatively, ROS or RNS can react directly with redox-sensitive protein thiols to form thiol radicals (P-S) or sulfenic acid (P-SOH) moieties on the protein, which can subsequently react with GSH to form the protein mixed disulfide (P-SSG) (Fig. 1B) (64). This process of protein glutathiolation has been recognized as a major post-translational regulator of protein function. Key proteins and cell-signaling factors regulated by glutathiolation have been reviewed elsewhere (64,65). Investigators have implicated mixed disulfide exchange reactions as a way to stabilize intracellular proteins, protect protein sulfhydryl moieties against irreversible oxidation to a sulfonyl group, and/or regulate enzymatic or signaling activities (65). Thus, the supramolecular organization of these proteins depends on the presence of exposed thiol moieties, and the modification of these groups by glutathiolation may be relevant to their function by either protecting them against further oxidation or preventing protein–protein cross-linking or polymerization.

**Formation of mixed disulfides with garlic-derived allylsulfide.** In recent studies (33,66,67) and in this article, we suggest that thiol–disulfide exchanges similar to that of protein glutathiolation can occur with physiological levels of S-cysteinyl compounds from garlic. Such interactions can produce a variety of effects on the activities of redox-sensitive signal transduction and pro-apoptotic proteins through S-cysteinylated proteins. That S-thiolation can produce a multiplicity of opposing effects on analogous proteins is exemplified by studies on the protein kinase C (PKC) family of enzymes (68). Accordingly, cysteine and other disulfide compounds (e.g., GSSG), under conditions where the activities of PKCy and PKCa are fully inactivated, actually stimulate the activity of PKCa. Furthermore, S-cysteinyl compounds affect the activity of PKCa only marginally or not at all. Thus, S-cysteinylated sites in signal proteins and transcription factors may be primary targets for development of chemopreventive or therapeutic agents that stimulate pro-apoptotic proteins or inactivate oncopgenic factors.

**Figure 1** Formation of glutathiolated proteins. (A) ROS, RNS, or glutathione peroxidase (GPOx) can oxidize GSH to form GSSG, which can undergo thiol-disulfide exchange with cysteinyl domains in proteins and result in the formation of a protein-mixed disulfide (P-SSG). (B) ROS and RNS can react directly with redox-sensitive protein thiols to form thiol radicals (P-S) or sulfenic acid (P-SOH) moieties on the protein, which can subsequently react with GSH to form the protein-mixed disulfide (P-SSG). Protein-SNO, S-nitrosylated protein; GSSG, protein-SNO, S-nitrosoglutathione.
Our data suggest that in rat-liver cytosol the

\[ \gamma \text{-cystathionase} \rightarrow R-S-S-H \text{ (e.g. SAMC)} \]

FIGURE 2 \( \gamma \)-Cystathionase formation of a persulfide fragment with cysteine S-mercapto conjugates from garlic. SAMC, S-propyl-

\( \gamma \)-cysteine, and S-penta-1,3-dienylmercapto-

cysteine are \( \beta \)-lyase substrates of \( \gamma \)-cystathionase, which catalyzes the elimination of a persulfide fragment, a source of sulfane sulfur through subsequent interactions with L-cysteine, GSSG, or protein disulfide.

There may be multiple mechanisms by which S-allylcysteiny1
derivatives exert their therapeutic effects. Several studies (69,70) suggest that S-allylcysteinyl compounds are metabolized to reactive intermediates such as allylmecapitan or allylhydro-
disulfide, and that these derivatives may react in situ with protein sulphydryls located on redox-sensitive proteins (20,33,66).

As an example of other allylsulfides (allylmethylsulfides, diallylsulfides, and dialilysulfides) have been detected in the breath of consumers of garlic and garlic supplements (71). Other transformations that occur both exogenously and endogenously in formation of mono-, di-, and triallylsulfanyl analogs as well as cysteinyl S-conjugates, such as SAMC, that can react with endogenous sulphydryl moieties, namely, glutathione, cysteine, coenzyme A, lipoic acid, and thiol-containing proteins within cells (71).

Enzyme-catalyzed transformations of cysteine S-conjugates. To identify the intracellular transformations involving allium-derived cysteine S-conjugates and their role as potential modifiers of redox-sensitive and/or transcription factors, we have examined whether these derivatives serve as substrates for enzymes that will convert these cysteine S-conjugates into metabolites capable of forming mixed disulfides with redox-sensitive proteins (Fig. 2). Cysteine S-conjugate \( \beta \)-lyase reactions catalyzed by pyridoxal \( 5^\prime \)-phosphate-dependent enzymes generate pyruvate, ammonium, and a reactive sulfur-containing fragment (RSH) (Eq. 1) most of which are theoretically capable of conjugating with cysteinyl moieties of proteins.

\[
\text{RSCH}_2\text{CH(CO}_2\text{)}\text{NH}_4^+ + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{C(O)CO}_2^+ + \text{NH}_4^+ + \text{RSH.} \tag{1}
\]

To date, our studies show that several cysteine S-conjugates isolated from garlic extracts serve as substrates for enzymes that can yield reactive sulfur intermediates. Mammalian enzymes that can catalyze \( \beta \)-lyase reactions with cysteine S-conjugates include kynureninase and several aminotransferases of which glutamine transaminase K (GTK) and mitochondrial aspartate amino-
transferase (mitAspAT) (72) are prominent. A \( \beta \)-lyase reaction can occur at the active site of an aminotransferase if the substrate contains a strong electrophilic-leaving group adjacent to the sulfur moiety. Previous studies with rat-liver cysteine that S-allylcysteine (SAC), SAMC, S-propylmercapto-
L-cysteine, and S-penta-1,3-dienylmercapto-
L-cysteine can undergo \( \beta \)-lyase 

reaction with garlic-derived cysteine \( \gamma \)-cystathionase (74). By contrast, S-cysteinyl conjugates with an allyl moiety (R = -CH\(_2\)CH\(_2\)CH\(_3\)) are substrates of the \( \beta \)-lyase reaction catalyzed by rat liver cystosol. Thus, the ability of rat-liver cystosol to catalyze a \( \beta \)-lyase reaction with SAC and SAMC as well as S-propyl- and S-pentadienyl-cysteine conjugates is consistent with the \( \beta \)-lyase specificity of \( \gamma \)-cystathionase.

To further support the finding that \( \gamma \)-cystathionase catalyzes \( \beta \)-elimination reactions with garlic-derived S-cysteinyl conjugates, we purified \( \gamma \)-cystathionase from rat liver using a modified procedure of Hargrove and Wichman (75) and found that \( \gamma \)-cystathionase activity using a standard substrate, L-homoserine, copurifies with \( \beta \)-lyase activity observed with SAMC. In brief, a 95 mL starting preparation of rat liver cytosol (40 g/L) was heated to 60°C, acidified to pH 5.2 with HCl, and centrifuged to remove precipitated protein. The supernatant fraction was neutralized to pH 7.0 with Tris base, brought to 50% saturation with ammonium sulfate and, after centrifugation, ammonium sulfate saturation was increased to 80%. The protein pellet was dissolved in a minimal amount of Tris-HCl pH 8.0 buffer to which ethanol was added to a final concentration of 80%. The pellet from the ethanolic solution was dissolved in phosphate buffer and eluted through a column of hydroxyapatite using increasing concentrations of phosphate buffer from 25 to 400 mM. The active fractions were pooled and subjected to gel filtration using a Sephacryl S-200 column equilibrated with phosphate buffer. The results of copurification of activities of SAMC \( \beta \)-lyase and \( \gamma \)-cystathionase are shown in Table 1.

Activity of allium-derived cysteine S-conjugates and their biological effects on redox-sensitive proteins. As an example of a \( \beta \)-lyase reaction on cystine, the products are initially pyruvate, ammonium, and thiocysteine (cysteine persulfide) (Eq. 2). Subsequent interaction of thiocysteine with unreacted cystine can generate cysteine and thiothione (cystine persulfide) (Eq. 3). At physiological pH, thiothione is converted to S-conjugates is due to \( \gamma \)-cystathionase. In studies using propargylglycine, an inhibitor of \( \gamma \)-cystathionase, \( \beta \)-lyase activity toward SAC, SAMC, S-propylmercapto-
L-cysteine, and S-penta-1,3-dienylmercapto-
L-cysteine was markedly diminished (67). In previous studies by others, purified rat-
liver \( \gamma \)-cystathionase was shown to catalyze \( \beta \)-elimination reactions with cysteine S-conjugates when the R-leaving group, as depicted in Eq. 1 above, is an alkyl moiety larger than ethyl (73). Accordingly, smaller members of the alkyl series, namely, S-methyl-L-cysteine and S-ethyl-L-cysteine, are not active with \( \gamma \)-cystathionase (74).

\[ \text{L-cystathionase (A) SAMC \( \beta \)-lyase (B) } \]

\[ \text{mU/mg protein} \]

\[
\begin{array}{llll}
\text{Homogenate} & 23.6 & 0.57 & 0.024 \\
\text{Heated extract} & 30.9 & 0.61 & 0.020 \\
\text{Acid fraction} & 54.6 & 1.39 & 0.025 \\
\text{(NH}_4\text{)}_2\text{SO}_4 \text{ fraction} & 70.4 & 1.41 & 0.020 \\
\text{Ethanol fraction} & 136 & 2.52 & 0.019 \\
\text{Hydroxyapatite} & 151 & 3.28 & 0.022 \\
\text{Sephacryl} & 2400 & 47.1 & 0.020 \\
\end{array}
\]

The reaction mixture (50 \( \mu \)L) contained 20 mmol/L L-homoserine (or 2.0 mM SAMC), 100 mmol/L potassium phosphate buffer (pH 7.2) and 10 \( \mu \)L of enzyme fraction. After incubation at 37°C for 10 min, \( \alpha \)-ketobutyrate (from homoserine) or pyruvate (from SAMC) was measured as the 2,4 dinitrophenyhydrazone derivative (67).
Liver cytosolic γ-cystathionase catalyzes the generation of reduced-sulfur species (sulfane sulfur) through a β-elimination with cystine (75). In a fashion similar to the generation of l-thiocysteine from cystine (l-cysteine persulfide), the β-lase reaction catalyzed by γ-cystathionase on allium-derived mercapto-l-cysteine S-conjugates could generate reactive persulfide (hydrodisulfide) species (RSSH). In vitro studies show that compounds containing persulfide or sulfur sulfane exhibit regulatory and antioxidant properties (76,77). The present finding that γ-cystathionase catalyzes β-lase reaction with SAMC, S-propyl-l-cysteine and S-penta-1,3-dienylmercapto-l-cysteine suggests that the eliminated sulfane sulfur compound may interact with endogenous disulfides such as l-cystine, GSSG, or protein disulfides. Distinct biological effects can occur through addition of sulfane sulfur to cysteinyl moieties on redox-sensitive proteins such as β-catenin, NFκB, PI3K, or P53 mediated by rhodanes, a widely distributed family of enzymes in animals. Rhodanes that display a 6-amino-acid loop with a cysteinyl moiety at the first position interact with substrates containing reactive sulfur or, in some cases, selenium (78). Figure 3 illustrates the possible mechanisms whereby rhodanes catalyzes the transfer of a sulfane sulfur atom from an anionic donor substrate to a reactive sulfur acceptor site by means of the persulfide group (Cys-SSH) on proteins.

Recent investigations have shown that rhodanese domains are structurally related to the catalytic subunit of Cdc25 phosphatase enzymes, and that these enzymes contain a nucleophilic catalytic cysteine residue that forms the initial persulfide linkage (78). The human Cdc25 family of phosphatases (Cdc25A, Cdc25B, and Cdc25C) regulates progression of cells through the cell cycle by dephosphorylating specific Cdk2/cyclin complexes particularly, Cdk2/CyclE and/or Cdk2/CyclA in G1/S, and Cdk2/CyclB during G2/M. S-Allylsulfide derivatives, in particular, diallylsulfides, can cause G2/M arrest of cells by activating p38 MAP kinase pathways with concomitant decrease in Cdc25C protein expression (79). Although Cdc25C appears to be the key mitotic regulator at the G2/M transition, it is Cdc25A and Cdc25B, which have been directly implicated in cancer and tumor development that may be targeted by S-cysteinyI metabolites. Thus, antiproliferative effects of garlic-derived S-alllysulfides may depend on their conversion to sulfane sulfur in tumor cells and/or to controlling activity of proliferative enzymes and signal factors through formation of reversible cysteinyl and/or disulfide transformations that occur in redox-regulated proteins (80).

**DISCUSSION**

Compelling evidence suggests that garlic constituents are effective inhibitors of tumor-cell proliferation, promotion, and progression. One notable effect of *allium* derivatives on normal and cancerous cells is their ability to enhance the endogenous antioxidant system, namely, GSH and detoxification pathways that include phase I (Cyp P450s) and phase II–conjugating enzymes (glucuronosyltransferases). Recent developments in understanding the mechanisms of chemopreventive agents, especially those formed by garlic, have focused on alterations in redox-regulated signal proteins and transcription factors. Many of these proteins exhibit active and inactive forms that differs by the oxidation state of highly conserved catalytic cysteine moieties. Accordingly, these sulphydryl centers are targets for intra- or intermolecular disulfide arrangements with neighboring protein-bound cysteinyl moieties or for mixed disulfide formation with cysteine, glutathione, or homocysteine. Our studies suggest that β-lase reactions are associated with activation of cysteinyl S-conjugates derived from *allium* foods into reactive persulfide or sulfane sulfur progenitors, which can also react with cysteinyl moieties on redox-sensitive proteins. We conclude that the allium-derived compounds with a mercapto moiety, such as SAMC, exert antiproliferative effects through the binding of sulfur-containing metabolites directly to redox-sensitive sites on signal proteins or transcription factors, thus arresting cells in mitosis and triggering activation of other pro-apoptotic–signaling pathways. The regulation of gene expression by ROS and antioxidants, as well as by determinants that can modify redox-sensitive signal proteins and transcription factors, is emerging as a novel theme in molecular medicine that has promising therapeutic implications. Evaluation of allium constituents in dietary strategies of chemoprevention must be considered in light of their biochemical transformations, interactions with endogenous organosulfur components, and reactivity with cysteinyl residues in proteins. Thus, more thorough study of the biochemistry of allium-derived cysteine S-conjugates may lead to new avenues for cancer prevention, treatment, and control.
LITERATURE CITED


