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Aminotransferase, L-amino acid oxidase and β-lyase reactions involving L-cysteine *S*-conjugates found in allium extracts Relevance to biological activity?

Arthur J.L. Cooper[a,b,*], John T. Pinto[c]

[a]*Departments of Biochemistry & Neurology and Neuroscience, Weill Medical College of Cornell University Medical College, New York, NY 10021, USA*
[b]*Burke Medical Research Institute, 785 Mamaroneck Avenue, White Plains, NY 10605, USA*
[c]*Institute for Cancer Prevention, Valhalla, NY 10595, USA*

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Abstract

Several cysteine *S*-conjugates that occur in extracts of garlic and other plants of the allium family possess anti-oxidant properties, and many, including *S*-allyl-L-cysteine (SAC) and *S*-allylmercapto-L-cysteine (SAMC), are promising anti-cancer agents. To understand possible biochemical mechanisms contributing to the protective effects, the ability of selected allium-derived L-cysteine *S*-conjugates to undergo various enzyme-catalyzed transformations was investigated. SAC, SAMC, *S*-propylmercapto-L-cysteine and *S*-penta-1,3-dienylmercapto-L-cysteine were shown to be substrates of: (a) highly purified rat kidney glutamine transaminase K (GTK); (b) purified snake venom L-amino acid oxidase; and (c) a cysteine *S*-conjugate β-lyase present in rat liver cytosol. *S*-Methylmercapto-L-cysteine was shown to be a substrate of GTK and L-amino acid oxidase, but not of the cysteine *S*-conjugate β-lyase. Evidence is presented that a major enzyme responsible for the cysteine *S*-conjugate β-lyase reactions in the rat liver cytosol is γ-cystathionase. The possible role of γ-cystathionase in generating sulfane sulfur from the disulfide-containing cysteine *S*-conjugates present in allium extracts, and the possible role of this sulfane sulfur in enzyme regulation, targeting of cancer cells and detoxification reactions is discussed.

An interesting side finding of the present work is that rat liver mitochondria are more active than rat liver cytosol in catalyzing a cysteine *S*-conjugate β-lyase reaction with the mitochondrial protoxicant *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC) at physiological pH and at low substrate concentration.

Keywords: *S*-Allyl-L-cysteine; *S*-Allylmercapto-L-cysteine; L-Amino acid oxidase; γ-Cystathionase; Cysteine *S*-conjugates; Glutamine transaminase K

1. Introduction

Epidemiological studies, clinical trials and animal models of chemical-induced carcinogenesis provide evidence for a protective role of allium vegetables against the development of a wide variety of cancers [1–3]. In particular, garlic contains the cysteine *S*-conjugate sulfoxide L-alliin [CH_2=$CHCH_2S(O)CH_2CH(NH_3^+)CO_2^-$], a non-odorous allylsulfinothiolated derivative of cysteine, that is transformed exogenously into several odorous allylpolysulfide analogues when the bulb is crushed, minced, or damaged [4] (Fig. 1). These bioactive components have been isolated from aqueous, ethanolic and fermented extracts of crushed garlic and have the potential to interact with a number of cellular targets, particularly those exhibiting reactive sulfhydryl moieties, whose functions range from control of cell cycle to expression of crucial anti-oxidant and detoxification enzymes [5–7]. Interactions with these processes may underlie garlic's putative therapeutic potential.

The ability of allylpolysulfides to act as pro-oxidants and to rapidly form *S*-conjugates with endogenous sulfhydryl compounds, such as L-cysteine and glutathione (GSH) is of

Abbreviations: DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; GTK, glutamine transaminase K; GSH, glutathione; GSSG, glutathione disulfide; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; SAC, *S*-allyl-L-cysteine; SAMC, *S*-allylmercapto-L-cysteine; TFEC, *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine

* Corresponding author. Tel.: +1 914 597 2437; fax: +1 914 594 2757.
E-mail address: acooper@burke.org (A.J.L. Cooper).

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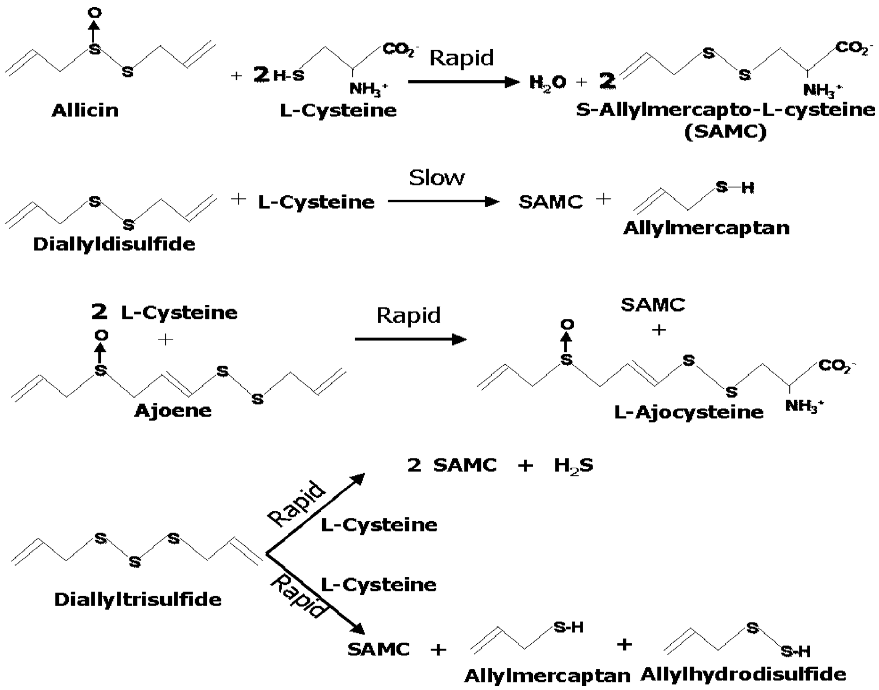

Fig. 1. Formation of *S*-allyl-L-mercaptocysteine (SAMC) from allium-derived polysulfides. The polysulfides are derived from L-alliin [CH_2=$CHCH_2S(O)CH_2CH(N^+H_3)CO_2^-$] in crushed or minced garlic preparations. The reactions with L-cysteine can take place within the lumen of the small intestine or intracellularly. Because of the high concentrations of GSH in most cells it is also possible that GSH can replace L-cysteine in the reactions shown, generating the corresponding allylmercapto glutathione *S*-conjugate. This compound presumably will be converted to the cysteine *S*-conjugate SAMC via enzymes of the γ-glutamyl cycle. Adapted from [7].

particular interest [8]. Since the intracellular concentration of GSH and cysteine are generally in the mM and 100 μM range, respectively (e.g. [9]), interactions with these endogenous compounds can alter the biological fate of the original allium derivative and affect redox balance within cells. Studies have shown that molecular transformations of several di- and triallylsulfide derivatives with dietary or intracellular cysteine result in formation of a water-soluble derivative, *S*-allylmercapto-L-cysteine (SAMC) [10] (Fig. 1). Although the exact mechanism by which allium derivatives exert their therapeutic effect is unknown, several studies suggest [5,11,12] that in situ formation of transported derivatives into an allylmercaptan or allylhydrodisulfide and subsequent reaction with reactive sulfhydryl moieties in redox sensitive proteins [13,14] may contribute to garlic's potential anti-cancer effect. Thus, objective evaluation of allium constituents in chemopreventive strategies must be considered in light of their biochemical transformations, interactions with endogenous organosulfur components and reactivity with cysteinyl residues in proteins.

On crushing of the garlic bulb, the endogenous enzyme, alliinase (a cysteine *S*-conjugate β-lyase) is activated and reacts with L-alliin, which is converted to allylsulfenic acid, pyruvate and ammonium (Fig. 2). Loss of water from two allylsulfenic acid molecules results in the formation of allicin. Allicin can react with L-cysteine derived from dietary proteins in the gastrointestinal tract to form the L-cysteine *S*-conjugate. As noted above, this compound can also be formed from a number of other L-alliin-derived polysulfides in the freshly crushed garlic extracts (Fig. 1). SAMC is one of several allium-derived cysteine *S*-conjugates formed in freshly crushed garlic.

Owing to their less odiferous nature, a variety of liquid and dried commercial preparations have gained popularity with consumers as dietary supplements [15–17]. These preparations contain several other cysteine *S*-conjugates, such as *S*-allyl-L-cysteine (SAC), *S*-methylmercapto-L-cysteine, *S*-propylmercapto-L-cysteine, *S*-penta-1,3-dienylmercapto-L-cysteine, and L-ajocysteine (Fig. 2).

As noted earlier, allicin [CH_2=CH–CH_2–$S(O)$–CH_2–CH=CH_2], a key metabolite of alliin, is formed by direct action of the enzyme alliinase (Figs. 1 and 2). Allicin was first isolated and its structure elucidated over 60 years ago [18,19]. Since that time, many studies have established allicin as a key ingredient contributing to the anti-microbial and medicinal properties of garlic extracts. In order to understand its mechanism of action an appreciation of its biochemical transformations in vivo is required. However, a problem with this approach is that allicin is thermally unstable and extremely labile in biological systems. For example, allicin is rapidly catabolized to allyl mercaptans within minutes after exposure to red blood cells [20]. Because of its lability, the anti-microbial and anti-cancer properties of allicin have been limited to studies employing cell culture conditions ([21] and references cited therein). Recently, however, Miron et al. [21], in addition to synthesizing [^{3}H]allicin for metabolic studies [22], have devised a

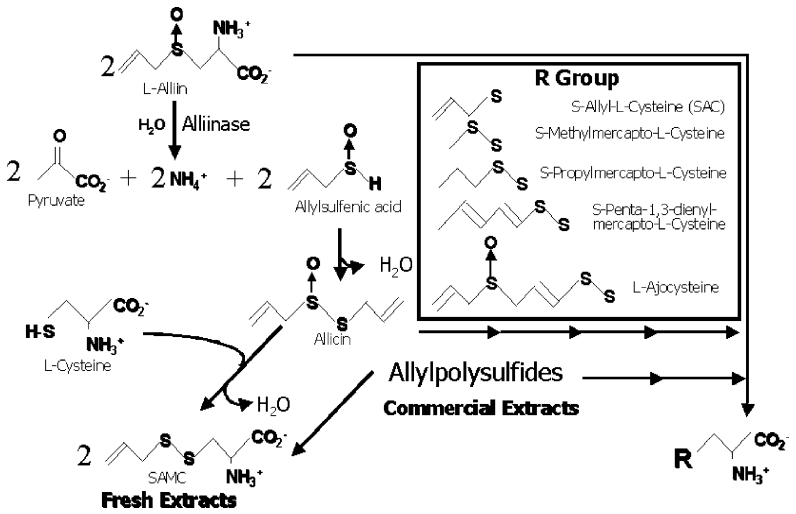

Fig. 2. Formation of cysteine *S*-conjugates in fresh garlic extracts and in aged extracts. The ability of each of the cysteine *S*-conjugates shown in the figure, except L-ajocysteine, to participate in aminotransferase, L-amino acid oxidase and cysteine *S*-conjugate β-lyase reactions is reported in the present work.

new in vivo approach to anti-microbial and anti-cancer therapy based on site-directed production of allicin. In this approach, the authors conjugated the enzyme alliinase to a monoclonal antibody (mAb) directed against ErbB2, a member of the epidermal growth factor receptor and a prognostic biomarker for advanced tumor types (breast, lung, ovarian, prostate, glioma, gastric, and squamous carcinoma of head and neck). After demonstrating that allicin is toxic to a number of mammalian tumor cells in culture, these investigators injected the mAb-alliinase conjugate into nude mice carrying the human tumor N87 (which expressed ErbB2). After injection of alliin, which is non-toxic, high anti-tumor activity was noted, while at the same time other tissues were unharmed, presumably as a result of the rapid clearance of allicin [21].

Methyallylsulfide [$CH_3SCH_2CH{=}CH_2$], diallylsulfide [$CH_2{=}CHCH_2SCH_2CH{=}CH_2$] and diallyldisufide [$CH_2{=}CHCH_2SSCH_2CH{=}CH_2$] have been detected in the breath of volunteers who have ingested garlic, and SAC has been detected in the plasma of these individuals [23]. These products undoubtedly arise in vivo via elimination reactions involving allicin, disulfide reductions/disulfide interchange reactions with GSH/cysteine and in one case (methylallylsulfide) methylation with *S*-adenosylmethionine [23]. However, the possible in vivo biochemical and direct enzymatic transformations of the alliin-derived L-cysteine *S*-conjugates have not been explored. As an aid to understanding the biological transformations involving allicin and its role as an anti-cancer agent, we have begun to investigate enzyme-catalyzed transformations of allium-derived allyl-containing L-cysteine *S*-conjugates. We suggest that an understanding of the biochemical interactions of these cysteine *S*-conjugates with redox sensitive signal and/or transcription factors will lead to further insights regarding the mechanisms by which the allium-derived compounds exert their biological effects. In support of our working hypothesis, we show that several cysteine *S*-conjugates found in fresh and commercial garlic extracts are substrates of purified glutamine transaminase K (GTK), purified snake venom L-amino acid oxidase, and a cysteine *S*-conjugate β-lyase in rat liver homogenates. The biological and chemotherapeutic implications are discussed.

2. Materials and methods

2.1. Reagents and enzymes

Ammediol [2-amino-2-methyl-1,3-propanediol], aminooxyacetate, D,L-propargylglycine [D,L-2-amino-4-pentynoic acid], L-methionine, L-phenylalanine, L-cysteine·HCl (solutions neutralized with NaOH before use), L-cystine, 2,4-dinitrophenylhydrazine, the sodium salts of pyruvate, 2-oxobutanoate, 2-oxoglutarate, and 2-oxo-4-methylthiobutanoate, *Crotalus adamanteus* L-amino acid oxidase (7.3 U/mg), catalase, semicarbazide·HCl (solutions neutralized with NaOH before use), were obtained from Sigma (St. Louis, MO). GTK (5 U/mg; 5 U/ml) was purified from the cytosolic fraction of rat kidney as described [24]. Recombinant rat liver mitochondrial aspartate aminotransferase (mitAspAT; 1.35 mg/ml; 410 U/mg) was a generous gift from Dr. Ana Iriarte University of Missouri-Kansas City. *S*-(1,1,2,2-Tetrafluoroethyl)-L-cysteine [TFEC; $CF_2HCF_2SCH_2CH(NH_3^+)CO_2^-$] and *S*-benzothiazolyl-L-cysteine (acetate salt) [$C_6H_4(SNC)SCH_2CH(NH_3^+)CO_2^-$], synthesized as previously described [25], were generously provided by Dr. Sam Bruschi, University of Washington, Seattle. SAC, SAMC, *S*-methylmercapto-L-cysteine, *S*-propylmercapto-L-cysteine and *S*-penta-1,3-dienylmercapto-L-cysteine were kindly supplied by Dr. Haru Amagase, Director of Research and Development, Wakunaga of America Co. Ltd., Mission Viejo, CA. Stock solutions of SAC, SAMC, *S*-methylmercapto-L-cysteine, and *S*-propyl-

mercapto-L-cysteine (2 mM in 100 mM potassium phosphate, pH 7.2) were stored at −20 °C. In the case of *S*-penta-1,3-dienylmercapto-L-cysteine, a 10 mM stock solution was made up in dimethylsulfoxide and stored at −20 °C.

2.2. Enzyme measurements

GTK, which is a freely reversible glutamine (methionine) aromatic amino acid aminotransferase [26], was measured by a slight modification of Cooper [24], adapted for well plate analysis. To determine the activity of GTK toward the sulfur-containing amino acids, 100 mM potassium phosphate buffer (pH 7.2) was used in place of the standard ammediol (pH 9.0) buffer. The lower pH was used because pH 7.2 is physiological and the *S*-cysteinyl compounds are expected to be unstable at pH 9.0. The reaction mixture (50 μl) contained 100 mM potassium phosphate buffer (pH 7.2), amino acid, 0.2 mM phenylpyruvate and GTK. After incubation at 37 °C, 150 μl of 1 M NaOH was added and the absorbance was read at 322 nm (ε 17,000 M^{-1} cm^{-1}). The amount of phenylpyruvate consumed was calculated from the decrease in absorbance relative to a blank consisting of complete reaction mixture plus boiled enzyme carried through the same procedure. The relative rate of GTK-catalyzed reactions at pH 7.2 versus pH 9.0 depends to some extent on the amino acid substrate, but the activity at pH 7.2 is generally about 30% that at pH 9.0 (the pH optimum with glutamine or phenylalanine as amino acid substrate).

L-Amino acid oxidase activity with various amino acid substrates (except *S*-benzothiazolyl-L-cysteine and L-cysteine) was measured by the procedure of Hafner and Wellner [27] modified for well plate analyses. The reaction mixture (200 μl) contained 100 mM potassium phosphate buffer (pH 7.2), 100 mM semicarbazide HCl (stock solutions previously neutralized with NaOH), L-amino acid, 10 μg (73 mU) of L-amino acid oxidase and catalase (10 U). The increase in absorbance due to appearance of 2-oxo acid semicarbazone was continuously monitored at 248 nm at a temperature of 37 °C. The extinction coefficients of typical 2-oxo acid semicarbazones (pyruvate, 2-oxoglutarate) at 248 nm are ∼10,000 [27,28]. The semicarbazones of the 2-oxo acids generated from the cysteine *S*-conjugates were assumed to have similar extinction coefficients at 248 nm. Under the present assay conditions, the formation of 2-oxo acid semicarbazones generated from each of the allium-derived cysteine *S*-conjugates was linear with time for at least 30 min. *S*-Benzothiazolyl-L-cysteine absorbs strongly at 248 nm and therefore the semicarbazone procedure cannot be used to measure L-amino acid oxidase activity with this amino acid. To measure L-amino acid oxidase activity with *S*-benzothiazolyl-L-cysteine the reaction mixture (50 μl) contained 100 mM potassium phosphate buffer (pH 7.2), 1.5–2.0 mM amino acid, 10 μg of L-amino acid oxidase and catalase (10 U). After incubation at 37 °C for 2 min, the reaction was terminated by addition of 20 μl of 5 mM 2,4-dinitrophenylhydrazine in 2 M HCl. After incubation at 37 °C for 5 min, 130 μl of 1 M NaOH was added and the absorbance of 2-oxo acid 2,4-dinitrophenylhydrazone at 430 nm was determined within 2 min against a blank consisting of 50 μl of reaction mixture containing boiled enzyme carried through the same procedure. The extinction coefficient of the 2,4-dinitrophenylhydrazone of the corresponding 2-oxo acid of *S*-benzothiazolyl-L-cysteine was 16,000 M^{-1} cm^{-1}. The extinction coefficient was determined by allowing the L-amino acid oxidase reaction on *S*-benzothiazolyl-L-cysteine to proceed to completion before addition of the 2,4-dinitrophenylhydrazine reagent.

To measure cysteine *S*-conjugate β-lyase activity, the reaction mixture (50 μl) contained L-amino acid, 100 mM potassium phosphate buffer (pH 7.2) and enzyme. After incubation at 37 °C, the reaction was terminated by addition of 20 μl of 5 mM 2,4-dinitrophenylhydrazine in 2 M HCl. After incubation at 37 °C for 10 min, 130 μl of 1 M NaOH was added and the absorbance of pyruvate 2,4-dinitrophenylhydrazone at 430 nm was determined (ε 15,000 M^{-1} cm^{-1}) within 2 min against a blank consisting of 50 μl of 100 mM potassium phosphate buffer (pH 7.2) plus enzyme carried through the same procedure. In some experiments, 0.1 mM 2-oxo-4-methylthiobutanoate or 0.1 mM 2-oxoglutarate was present in the assay mixture resulting in a slightly elevated blank absorbance. However, the presence of 0.1 mM 2-oxo acid had no effect on the slope of the standard curve for pyruvate. When crude liver cytosol or mitochondrial homogenates were used it was necessary to briefly centrifuge the samples (2 min at 10,000 × *g*) to remove insoluble material before measuring the absorbance at 430 nm. In control experiments, it was shown that none of the β-lyase substrates incubated at 37 °C for 1 h at a concentration of 1.5 mM in 100 mM potassium phosphate buffer (pH 7.2) interfered with the 2,4-dinitrophenylhydrazone reactions. On the other hand, incubation of 5 μl of liver supernatant fraction or 10 μl of liver mitochondria in 50 μl of 100 mM potassium phosphate buffer (pH 7.2) for 1 h at 37 °C resulted in a slight increase in absorbance (0.04–0.05) at 430 nm in the 2,4-dinitrophenylhydrazine assay. Since most of the incubations were carried out for 20 min, the absorbance correction due to formation of 2,4-dinitrophenylhydrazine-reactive material was small (∼0.015) in the blank containing homogenate but lacking β-lyase substrate. It is important to note that where transamination of the allium-derived cysteine *S*-conjugates competes with the β-lyase reaction, the net loss of 2-oxo-4-methylthiobutanoate (or 2-oxoglutarate) will be offset by the stoichiometric increase in the 2-oxo analogue of the cysteine *S*-conjugate. Although the extinction coefficients of the 2,4-dinitrophenylhydrazones of the 2-oxo acid analogues of the cysteine *S*-conjugates were not determined, in our experience the extinction coefficients of pyruvate, 2-oxo-4-methylthiobutanoate,

2-oxoglutarate and 2-oxobutanoate 2,4-dinitrophenylhydrazones at 430 nm under basic conditions are similar (ε ~15,000–16,000). Therefore, inasmuch as transamination results in no net 2-oxo acid formation, the increase in absorbance at 430 nm was considered a reliable indicator of pyruvate formation via a β-lyase reaction, even in the presence of competing transamination.

γ-Cystathionase activity was measured using L-homoserine (e.g. [29]) as an alternative substrate to L-cystathionine. A low concentration of homoserine was chosen for the assay for comparison with the allium-derived cysteine *S*-conjugates, which are generally only sparingly soluble in aqueous buffers. The reaction mixture (50 μl) contained 1.5 mM L-homoserine, 100 mM potassium phosphate buffer (pH 7.2) and enzyme. After incubation at 37 °C, the reaction was terminated by the addition of 5 mM 2,4-dinitrophenylhydrazine in 2 M HCl. After incubation at 37 °C for 10 min, 130 μl of 1 M NaOH was added and the absorbance of 2-oxobutanoate 2,4-dinitrophenylhydrazone at 430 nm was determined (ε 15,000 M^{-1} cm^{-1}) within 2 min against a blank consisting of 50 μl of buffer plus enzyme carried through the same procedure.

All spectrophotometric determinations were carried out in a SpectraMax 96-well plate spectrophotometer (Molecular Devices, Sunnyvale, CA).

2.3. Preparation of rat liver fractions

Cytosol (40 mg of protein/ml) and highly purified mitochondria (48 mg of protein/ml) were prepared from the livers of 6-month-old male Fisher 344 × Brown Norway F1 rats by the method of Cooper et al. [30]. The freshly prepared mitochondria when kept on ice were stable (i.e. maintained respiration in the presence of suitable substrates) for over 24 h after isolation. The cytosolic and mitochondrial fractions were stored at −20 °C.

2.4. Statistics

For determinations where n is >3, the mean ± the standard error of the mean (S.E.M.) is reported. Statistical comparisons were carried out using the Mann–Whitney *U*-test; a p-value ≤0.05 was considered significant.

3. Results

3.1. Cysteine S-conjugates are aminotransferase substrates of rat kidney GTK

Rat kidney GTK exhibits a remarkably high affinity for phenylpyruvate as the 2-oxo acid substrate (~20 μM) [26]. Based on the high binding affinity and high molar extinction coefficient of phenylpyruvate enol under basic conditions (see Section 2), we devised a sensitive assay for measuring phenylpyruvate disappearance at a low concentration (200 μM) of this 2-oxo acid substrate in the presence of various L-cysteine *S*-conjugates and GTK (Table 1). GTK activities toward L-glutamine, L-methionine, L-cysteine and L-cystine were measured for comparison. L-Cysteine is a moderately good substrate of rat kidney GTK [26], and cystine is a very good aminotransferase substrate of bovine and rat kidney GTK (reviewed in [31]). However, the previous assays were carried out at the non-physiological pH values of 8.5–9.0. Table 1 shows that L-cysteine and L-cystine are aminotransferase substrates of rat kidney GTK at the more physiological pH of 7.2. Under conditions shown in Table 1, the allium-derived L-cysteine *S*-conjugates are about 5–15% as effective as L-glutamine as aminotransferase substrates and about 15–45% as effective as L-methionine. The allium-derived L-cysteine *S*-conjugates are more effective than L-cysteine, but less effective than L-cystine, as aminotransferase substrates.

Table 1
Rat kidney GTK-catalyzed transamination of phenylpyruvate with L-glutamine, L-methionine, L-cystine, L-cysteine and L-cysteine *S*-conjugates[a]

Transaminase substrate	GTK added (mU)	Time of incubation (min)	Phenylpyruvate consumed (nmol)
L-Glutamine	1	10	1.39 ± 0.02
L-Methionine	1	30	1.78 ± 0.05
L-Cystine (0.15 mM)	2.5	10	1.53 ± 0.08
L-Cysteine	2.5	90	1.25 ± 0.05
S-Allyl-L-cysteine (SAC)	2.5	60	2.68 ± 0.13
S-Methylmercapto-L-cysteine	2.5	60	2.28 ± 0.25
S-Allylmercapto-L-cysteine (SAMC)	2.5	60	3.18 ± 0.10
S-Propylmercapto-L-cysteine	2.5	60	2.78 ± 0.33
S-Penta-1,3-dienylmercapto-L-cysteine (0.5 mM)	1.25	60	1.03 ± 0.1
S-(1,1,2,2-Tetrafluoroethyl)-L-cysteine (TFEC)	1	30	1.80 ± 0.22
S-Benzothiazolyl-L-cysteine	5	60	N.D.

[a] The reaction mixture (50 μl) contained 100 mM potassium phosphate buffer (pH 7.2), 1 mM L-amino acid (except where indicated), 0.2 mM phenylpyruvate (10 nmol) and GTK. The reaction mixture was incubated at 37 °C for the indicated time. Thereafter, 150 μl of 1 M NaOH was added and the absorbance was read at 322 nm. The amount of phenylpyruvate consumed was calculated from the decrease in absorbance relative to a blank consisting of complete reaction mixture plus boiled enzyme carried through the same procedure. Owing to the very high affinity of GTK for phenylpyruvate, the disappearance of 0.2 mM phenylpyruvate in the presence of 1 mM L-glutamine is linear until at least 90% of the 2-oxo acid is consumed. In separate experiments this was also confirmed for L-methionine, SAC and SAMC (data not shown). Purified GTK is stable in 100 mM potassium phosphate buffer (pH 7.2) for at least 2 h at 37 °C. Therefore, it is reasonable to assume that the data shown are indicative of relative rates. N.D., not detectable; N = 3.

The toxic halogenated cysteine *S*-conjugate TFEC is often used as a model substrate of various cysteine *S*-conjugate β-lyases (e.g. [32]) (see Section 4). TFEC is a particularly good β-lyase substrate of rat kidney GTK [33]. However, transamination competes with β elimination at the active site [33,34] (see Section 4). Table 1 shows that TFEC is about as effective as methionine as an aminotransferase substrate of GTK. Although *S*-benzothiazolyl-L-cysteine has also been used as a model cysteine *S*-conjugate β-lyase substrate [32], this compound is not a β-lyase substrate of GTK [33].

Table 1 illustrates that *S*-benzothiazolyl-L-cysteine is also not an aminotransferase substrate of GTK.

3.2. Rat kidney GTK catalyzes a cysteine S-conjugate β-lyase reaction with S-penta- 1,3-dienylmercapto-L-cysteine

To determine whether rat kidney GTK can catalyze a β-lyase reaction with the allium-derived L-cysteine *S*-conjugates, each compound was incubated with 15 mU of enzyme and 0.2 mM phenylpyruvate in the 0.05 ml reaction mixture (pH 7.2) for 2 h at 37 °C. Little or no pyruvate (≤0.5 nmol) could be detected when 1 mM SAMC, SAC, *S*-methylmercapto-L-cysteine or *S*-propylmercapto-L-cysteine was present in the reaction mixture. By contrast, a significant amount of pyruvate (5.09 ± 0.64 nmol; $n = 6$) was produced when 0.5 mM *S*-penta-1,3-dienylmercapto-L-cysteine was used as a substrate. The amount of pyruvate generated from 1.0 mM TFEC in 30 min in the presence of 10 mU of GTK, 0.2 mM phenylpyruvate, and 100 mM phosphate (pH 7.2) was 16.0 ± 1.2 nmol ($n = 3$). Comparisons of these data with those in Table 1 show that in the presence of 1.0 mM TFEC (pH 7.2) and 0.2 mM phenylpyruvate, GTK catalyzes β-lyase and aminotransferase reactions with about equal efficacy. By contrast, in the presence of 0.5 mM *S*-penta-1,3-dienylmercapto-L-cysteine, GTK preferentially catalyzes transamination over β-elimination by a factor of about 5.

3.3. Cysteine S-conjugates are substrates of L-amino acid oxidase

Snake venom L-amino acid oxidase has wide substrate specificity. Generally, L-amino acids possessing a hydrophobic group, such as L-methionine and L-phenylalanine are the most active substrates (e.g. [35]). Among the amino acids commonly found in proteins, L-methionine is the best substrate [35]. Table 2 compares the activities of the cysteine *S*-conjugates with L-methionine as substrates of L-amino acid oxidase. SAC is as effective as methionine as a substrate, most likely because the size of the hydrophobic group is quite similar. Other allium-derived L-cysteine *S*-conjugates are also substrates of L-amino acid oxidase. The mixed disulfide *S*-methylmercapto-L-cysteine is very effective as a substrate, but the effectiveness falls off as the size of the alkyl group in the cysteine mixed disulfide decreases in the order: propyl > allyl > methyl (Table 2). *S*-Benzothiazolyl L-cysteine and TFEC are also excellent substrates (Table 2). In fact, *S*-benzothiazolyl-L-cysteine, which contains a large aromatic group attached at the sulfur, is an even better substrate of snake venom L-amino acid oxidase than is L-methionine.

Table 2
Oxidation of L-glutamine, L-cystine, L-cysteine, L-methionine and various L-cysteine *S*-conjugates by L-amino acid oxidase[a]

Substrate	Rate (nmol/min)
L-Glutamine	0.68 ± 0.02
L-Methionine	6.88 ± 0.12
L-Cystine (0.15 mM)	2.40 ± 0.01
L-Cysteine	2.80 ± 0.12
S-Allyl-L-cysteine	6.52 ± 0.48
S-Methylmercapto-L-cysteine	4.64 ± 0.32
S-Allylmercapto-L-cysteine	1.79 ± 0.20
S-Propylmercapto-L-cysteine	1.13 ± 0.05
S-Penta-1,3-dienylmercapto-L-cysteine (0.5 mM)	0.50 ± 0.10
S-(1,1,2,2-Tetrafluoroethyl)-L-cysteine	5.56 ± 0.01
S-Benzothiazolyl-L-cysteine	11.1 ± 0.2

[a] The reaction mixture (200 μl) contained 1.5 mM L-amino acid (except where noted), 100 mM semicarbazide HCl (except where noted), 10 μg (73 mU) of L-amino acid oxidase, 10 U of catalase, and 100 mM potassium phosphate buffer (pH 7.2). The increase in absorbance at 248 nm was continuously recorded at 37 °C against a blank consisting of complete reaction mixture lacking L-amino acid oxidase. Semicarbazide was omitted when *S*-benzothiazolyl-L-cysteine was used as substrate. 2-Oxo acid formation from this amino acid was measured by the 2,4-dinitrophenylhydrazone procedure (see Section 2). $N = 3$.

3.4. Rat liver cytosol catalyzes β-elimination reactions with allium-derived L-cysteine S-conjugates

Table 3 shows that the allium-derived L-cysteine *S*-conjugates investigated here, with the exception of *S*-methylmercapto-L-cysteine, are effective β-lyase substrates of an enzyme present in rat liver cytosol. Except in the case of *S*-penta-1,3-dienylmercapto-L-cysteine the activity is not stimulated by 0.1 mM 2-oxo-4-methylthiobutanoate. Activities in liver homogenates with SAC and SAMC are strongly inhibited by aminooxyacetate and by D,L-propargylglycine (Table 3). Under the conditions shown in Table 3, the best cysteine *S*-conjugate β-lyase substrate among the allium-derived compounds is SAMC, which is about half as effective as the prototypical cysteine *S*-conjugate β-lyase substrates, TFEC and *S*-benzothiazolyl-L-cysteine (see Section 4). For completeness, the ability of the rat liver cytosol to catalyze a γ-elimination reaction with L-homoserine—a property of γ-cystathionase—was determined. The allium-derived compounds are about 2–8% as effective (as β-lyase substrates) relative to L-homoserine (as a γ-lyase substrate) (Table 3).

If 2-oxo acids were produced from the allium-derived L-cysteine *S*-conjugates by an L-amino acid oxidase reaction then this would lead to an overestimation of pyruvate formation by the β-lyase reaction catalyzed by rat liver

Table 3
γ-Cystathionase (L-homoserine as substrate) and β-lyase activities with L-cystine, L-cysteine and various L-cysteine *S*-conjugates catalyzed by rat liver cytosol[a]

	Minus propargylglycine	Plus propargylglycine
2-Oxobutanoate formed (nmol)		
L-Homoserine (0.5 μl of cytosol)	11.5 ± 0.7	N.D.[b]
Pyruvate formed (nmol)		
L-Cystine (0.15 mM)	3.83 ± 0.20	N.D.[b]
L-Cysteine	3.13 ± 0.65	1.40 ± 0.18[b]
S-Allyl-L-cysteine	1.71 ± 0.48	0.25 ± 0.10[b]
S-Allyl-L-cysteine + 0.1 mM 2-oxo-4-methylthiobutanoate	1.83 ± 0.32	–[c]
S-Allyl-L-cysteine + 1 mM aminooxyacetate	0.22 ± 0.05b	–
S-Allylmercapto-L-cysteine	7.23 ± 0.85	1.76 ± 0.12[b]
S-Allylmercapto-L-cysteine + 0.1 mM 2-oxo-4-methylthiobutanoate	8.30 ± 0.35	–
S-Allylmercapto-L-cysteine + 1 mM aminooxyacetate	0.35 ± 0.10b	–
S-Methylmercapto-L-cysteine	N.D.	N.D.
S-Propylmercapto-L-cysteine	3.70 ± 1.01	N.D.[b]
S-Propylmercapto-L-cysteine+ 0.1 mM 2-oxo-4-methylthiobutanoate	4.20 ± 0.52	–
S-Penta-1,3-dienylmercapto-L-cysteine (0.5 mM)	4.15 ± 1.00	1.43 ± 0.05[b]
S-Penta-1,3-dienylmercapto-L-cysteine (0.5 mM) + 0.1 mM 2-oxo-4-methylthiobutanoate	5.92 ± 0.42[d]	–
S-(1,1,2,2-Tetrafluoroethyl)-L-cysteine	16.3 ± 1.9	12.1 ± 1.3[b]
S-(1,1,2,2-Tetrafluoroethyl)-L-cysteine + 0.1 mM 2-oxo-4-methylthiobutanoate	23.2 ± 1.3[d]	–
S-Benzothiazolyl-L-cysteine	15.2 ± 1.4	5.76 ± 0.58[b]
S-Benzothiazolyl-L-cysteine + 0.1 mM 2-oxo-4-methylthiobutanoate	13.4 ± 0.8	–

[a] The reaction mixture (50 μl) contained 1.5 mM L-amino acid (except where indicated), 100 mM potassium phosphate buffer (pH 7.2), 5 μl (0.2 mg of protein) of rat liver cytosol (except where indicated), and where indicated, 0.1 mM 2-oxo-4-methylthiobutanoate, 1 mM aminooxyacetate or 10 mM D,L-propargylglycine. After incubation at 37 °C for 20 min, pyruvate (or 2-oxobutanoate in the case of L-homoserine) was measured. N.D.: not detectable; $N = 3$.
[b] Significantly different from activity in the absence of added inhibitor (aminooxyacetate or propargylglycine) with $p = 0.05$.
[c] Indicates not determined.
[d] Significantly different from the no added 2-oxo-4-methylthiobutanoate value with $p = 0.05$.

cytosol [the 2,4-dinitrophenylhydrazone procedure cannot distinguish between pyruvate and other 2-oxo acids]. Aminooxyacetate is a PLP antagonist that should not affect the L-amino acid oxidase reaction. Thus, the almost complete inhibition of the production of 2,4-dinitrophenylhydrazone-forming material from SAC and SAMC by aminooxyacetate (Table 3) suggests that most, if not all, the 2,4-dinitrophenylhydrazone-forming material is derived from a PLP-containing enzyme and not from an L-amino acid oxidase reaction. Moreover, mammalian L-amino acid oxidase has a much higher pH optimum (>9.0) than the pH used in the measurement of cysteine *S*-conjugates β-lyase activities (pH 7.2) [36].

3.5. Rat liver mitochondria catalyze a weak β-elimination reaction with SAMC

γ-Cystathionase is a strictly cytosolic enzyme in rat liver [37]. Therefore, γ-cystathionase activity may be used as an indicator of the purity of the liver mitochondria used in the present studies. Table 4 shows that the purified rat liver mitochondria contain very little γ-cystathionase activity relative to that in the cytosolic fraction. The activity in the mitochondrial fraction is close to the limit of detection. The specific activity of γ-cystathionase (1.5 mM L-homoserine as substrate) in the mitochondria is <0.2% that exhibited by the cytosolic fraction (compare data in Tables 3 and 4). This finding is evidence that there is little or no contamination of the mitochondrial fraction with cytosol. The mitochondria exhibit some β-lyase activity toward SAMC, but the specific activity is less than that exhibited with the cytosolic fraction (compare Tables 3 and 4). This activity is not significantly affected by the addition of 0.1 mM 2-oxo-4-methylthiobutanoate, 0.1 mM 2-oxoglutarate or 10 mM D,L-propargylglycine, but is inhibited by 1 mM aminooxyacetate (Table 4). β-Lyase activity toward TFEC and *S*-benzothiazolyl-L-cysteine in the mitochondrial fraction is much more prominent than toward SAMC. β-Lyase specific activities with TFEC and *S*-benzothiazolyl-L-cysteine as substrates are also greater in the mitochondria than in the cytosolic fraction (compare data in Tables 3 and 4). The β-lyase activity toward TFEC in the mitochondria is strongly activated by 0.1 mM 2-oxo-4-methylthiobutanoate, but less so by 0.1 mM 2-oxoglutarate (Table 4). Propargylglycine has no effect on the β-lyase reaction with TFEC, but significantly inhibits the β-lyase reaction with *S*-benzothiazolyl-L-cysteine (Table 4). The findings show that the allium-derived cysteine *S*-conjugates investigated here, with the exception of SAMC, are metabolized to pyruvate exclusively in the cytosolic fraction of rat liver. However, even with SAMC, the ability of highly purified rat liver mitochondria to catalyze conversion of this amino acid to pyruvate is limited compared to the cytosolic fraction. By contrast, under the conditions used in the present assay, the prototypical cysteine *S*-conjugate β-lyase substrates TFEC and *S*-benzothiazolyl-L-cysteine are more readily converted to pyruvate by the mitochondrial fraction than by the cytosolic fraction (see Section 4).

Table 4
γ-Cystathionase (L-homoserine as substrate) and β-lyase activities with cysteine *S*-conjugates catalyzed by rat liver mitochondria[a]

	Time of Incubation (min)	2-Oxobutanoate formed (nmol)
L-Homoserine	60	0.45 ± 0.03
L-Homoserine plus propargylglycine	60	N.D.[b]

	Time of Incubation (min)	Pyruvate formed (nmol)		
		No added 2-oxo acid	Plus OMTB	Plus OG
S-Allylmercapto-L-cysteine	60	0.99 ± 0.18	1.18 ± 0.18	1.13 ± 0.10
S-Allylmercapto-L-cysteine plus propargylglycine	60	1.23 ± 0.30	–[d]	–
S-Allylmercapto-L-cysteine plus aminooxyacetate	60	N.D.[b]	–	–
S-(1,1,2,2-Tetrafluoroethyl)-L-cysteine	5	24.9 ± 1.3	39.7 ± 0.9[c]	28.8 ± 0.4[c]
S-Benzothiazolyl-L-cysteine	5	9.75 ± 0.15	9.33 ± 0.85	10.2 ± 0.4

[a] The reaction mixture (50 μl) contained 1.5 mM L-homoserine (or L-cysteine *S*-conjugate), 100 mM potassium phosphate buffer (pH 7.2), 10 μl (0.48 mg of protein) of rat liver mitochondria, and, where indicated, 0.1 mM 2-oxo-4-methylthiobutanoate (OMTB), 0.1 mM 2-oxoglutarate (OG), 1 mM aminooxyacetate, or 10 mM D,L-propargylglycine. After incubation at 37 °C for the time indicated, pyruvate (or 2-oxobutanoate in the case of L-homoserine) was measured. N = 3–6. N.D., not detected. No pyruvate formation (<0.5 nmol) could be detected when *S*-allylmercapto-L-cysteine was replaced with 1.5 mM *S*-allyl-L-cysteine, 1.5 mM *S*-methylmercapto-L-cysteine, 1.5 mM *S*-propylmercapto-L-cysteine or 0.2 mM *S*-penta-1,3-dienylmercapto-L-cysteine.
[b] Significantly different from the no added inhibitor value with $p = 0.05$.
[c] Significantly different from the no added 2-oxo acid value with $p = 0.05$.
[d] Indicates not determined.

4. Discussion

4.1. Cysteine S-conjugate β-lyase reactions catalyzed by rat liver enzymes

Several PLP-containing enzymes catalyze β-lyase side reactions with cysteine *S*-conjugates, such as TFEC, *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) and *S*-benzothiazolyl-L-cysteine. The cysteine *S*-conjugate β-lyase reaction generates pyruvate, ammonium and a sulfur-containing fragment (Eq. (1)). Mammalian cysteine *S*-conjugate β-lyases include kynureninase and several aminotransferases. Aminotransferases that are the most active as in vitro β-lyases (with TFEC as substrate) include GTK [33] and mitochondrial aspartate aminotransferase [38] (for reviews see for example [31,32]).

$$\mathrm{RSCH_2CH(CO_2^-)NH_3^+ + H_2O \rightarrow CH_3C(O)CO_2^- + NH_4^+ + RSH} \quad (1)$$

Because a large number of sulfur-containing amino acids, including several halogenated L-cysteine *S*-conjugates, L-homocysteine *S*-conjugates, L-cysteine and L-cystine, are aminotransferase substrates of GTK (reviewed in [31,32]), we considered the possibility that the cysteine *S*-conjugates found in allium extracts might also be aminotransferase substrates of GTK. Moreover, if sufficient electron withdrawal capacity resides adjacent to the sulfur moiety, a β-lyase reaction might compete with transamination at the active site. In the present work, we show that the allium-derived cysteine *S*-conjugates are moderately good aminotransferase substrates of GTK (Table 1). However, of the allium-derived L-cysteine *S*-conjugates investigated here, only *S*-penta-1,3-dienylmercapto-L-cysteine was effective both as an aminotransferase and β-lyase substrate of GTK. Presumably, the conjugated double bond system in *S*-penta-1,3-dienylmercapto-L-cysteine provides enough electron-withdrawal at the cysteinyl sulfur moiety to facilitate a β-elimination reaction with GTK. However, even in this case the aminotransferase reaction is favored over the β-lyase reaction.

Table 3 shows that the allium-derived cysteine *S*-conjugates SAC, SAMC, *S*-propylmercapto-L-cysteine, and *S*-penta-1,3-dienylmercapto-L-cysteine undergo β-elimination in the presence of rat liver cytosol. The present study clearly shows that, except for some β-lyase activity toward *S*-penta-1,3-dienylmercapto-L-cysteine, this β-lyase activity cannot be due to GTK. Our data suggest that the bulk of the β-lyase activity toward the allium-derived L-cysteine *S*-conjugates in the rat liver cytosolic fraction is due to γ-cystathionase. Only a small amount of β-lyase activity toward SAMC was detected in rat liver mitochondria, and no activity was detected with the other allium-derived L-cysteine *S*-conjugates in these organelles (Table 4). A major cysteine *S*-conjugate β-lyase of rat liver mitochondria with TFEC as substrate is mitochondrial aspartate aminotransferase [38]. However, no β-lyase activity toward SAMC could be detected with highly purified rat liver mitochondrial aspartate aminotransferase (data not shown). Data in Table 4 show that γ-cystathionase is not responsible for the β-lyase activity toward SAMC in the liver mitochondria. The identity of the mitochondrial SAMC β-lyase remains to be identified. Because the β-lyase activity toward SAMC in the mitochondria is stimulated by 2-oxo-4-methylthiobutanoate (Table 4), an aminotransferase is a likely candidate.

In the present study, at the physiological pH value of 7.2 and at the relatively low concentration of cysteine *S*-conjugate (1.5 mM), the specific activity of cysteine *S*-conjugate β-lyase with TFEC as substrate is several-fold higher in the mitochondria than in the cytosol (compare Tables 3 and 4). The higher activity in liver mitochondria

than in liver cytosol was not recognized previously, possibly because higher pH values (8.0–9.0) and higher concentrations (5–10 mM) of TFEC were commonly used in assays of β-lyase activity toward this cysteine *S*-conjugate. The present finding is interesting because TFEC is a mitochondrial protoxicant. In the mitochondria, cysteine *S*-conjugate β-lyases, including mitochondrial aspartate aminotransferase, convert TFEC to a toxicant (a sulfur-containing fragment that thioacylates protein lysyl residues) (reviewed in [32]).

4.2. Evidence that the β-lyase activity toward allium-derived cysteine S-conjugates is due to cytosolic γ-cystathionase

The PLP-dependent enzyme γ-cystathionase catalyzes a β-elimination reaction with L-cystine (e.g. [39–41]). Cystine, together with most of the allium-derived L-cysteine *S*-conjugates studied here, may be regarded as belonging to a special class of cysteine *S*-conjugates depicted as $RSSCH_2CH(CO_2^-)NH_3^+$. This consideration suggests that at least part of the β-lyase activity in rat liver cytosol directed toward the allium-derived cysteine *S*-conjugates (Table 3) may be due to γ-cystathionase. Evidence in support of this hypothesis is presented below.

Propargylglycine is an effective K_{cat} inhibitor of γ-cystathionase [29]. Although this compound is not completely selective for γ-cystathionase, the fact that the β-lyase activity noted with SAC, SAMC, *S*-propylmercapto-L-cysteine and *S*-penta-1,3-dienylmercapto-L-cysteine is strongly inhibited by propargylglycine (Table 3) is in accord with the idea that the β-lyase activity toward the allium-derived L-cysteine *S*-conjugates in the liver cytosol is due at least in part to γ-cystathionase. Moreover, purified rat liver γ-cystathionase was previously shown to catalyze β-elimination reactions with cysteine *S*-conjugates [$RSCH_2CH(CO_2^-)\ NH_3^+$, Eq. (1)] when R is an alkyl group larger than ethyl [42]. The active site must be relatively large because cysteine *S*-conjugates where R is a bulky alkane [*S*-(cyclohexyl)-L-cysteine, *S*-(*tert*-butyl)-L-cysteine] are substrates [42]. The enzyme is not active with the smaller members of the series, namely *S*-methyl-L-cysteine and *S*-ethyl-L-cysteine, but is active with *S*-propyl-L-cysteine [42]. SAC ($R = -CH_2CH{=}CH_2$), which is almost identical in size to *S*-propyl-L-cysteine ($R = -CH_2CH_2CH_3$), is a substrate of the β-lyase reaction catalyzed by rat liver cytosol (Table 4). However, *S*-methylmercapto-L-cysteine ($R = -SCH_3$) is not a detectable β-lyase substrate of the rat liver cytosol preparation (Table 3). This compound is similar in size to *S*-ethyl-L-cysteine ($R = -CH_2CH_3$). Thus, the ability of rat liver cytosol to catalyze a β-lyase reaction with SAC, but not with *S*-methylmercaptocysteine, is fully consistent with the β-lyase specificity of γ-cystathionase. Finally, 2-oxo-4-methylthiobutanoate does not significantly stimulate the rat liver cytosol-catalyzed β-elimination of SAC, SAMC, and *S*-propyl-L-cysteine. The 2-oxo acid does, however, stimulate the β-lyase activity with *S*-penta-1,3-dienylmercapto-L-cysteine and TFEC (Table 3). The finding that TFEC is a β-lyase substrate in rat liver cytosol is in accord with GTK and other aminotransferases catalyzing a β-lyase reaction with this halogenated cysteine *S*-conjugate in this tissue fraction. However, our data support the idea that β-lyase activity toward the allium-derived cysteine *S*-conjugates in rat liver cytosol is not likely contributed by GTK or a GTK-like aminotransferase, except perhaps in the case of *S*-penta-1,3-dienylmercapto-L-cysteine.

4.3. Possible biological effects associated with 2-oxo acid formation from allium-derived L-cysteine S-conjugates

A number of sulfur-containing amino acids were previously shown to be excellent substrates of snake venom L-amino acid oxidase (e.g. [43]). We have extended these findings to allium-derived L-cysteine *S*-conjugates (Table 2). Rat liver has long been known to possess L-amino acid oxidase activity [36], now known to be a property of the A form of L-α-hydroxy acid oxidase. A partially purified preparation of L-amino acid oxidase from rat liver was previously shown to catalyze the oxidation of a halogenated cysteine *S*-conjugate and two non-halogenated cysteine *S*-conjugates (*S*-benzyl-L-cysteine and *S*-phenyl-L-cysteine) to the corresponding 2-oxo acids at pH 8.6 [44]. However, as noted above, we found little evidence that an L-amino acid oxidase reaction operates in rat liver cytosol at pH 7.2 in the case of SAC and SAMC (Table 4 and Section 3). The L-amino acid oxidase of rat liver has a very high pH optimum (∼10.0), exhibiting low activity at pH 7.2 [36]. Therefore, it seems probable that 2-oxo acids of the corresponding L-cysteine *S*-conjugates in mammalian liver would arise from aminotransferase reactions, such as that catalyzed by GTK, rather than from an L-amino acid oxidase reaction. Nevertheless, snake venom L-amino acid oxidase should prove useful in providing 2-oxo acid standards for more detailed future studies of aminotransferase/β-lyase reactions involving allium-derived L-cysteine *S*-conjugates.

Our studies show that cysteine *S*-conjugates found in allium extracts are moderately good aminotransferase substrates of purified GTK. Previously, it was shown that the 2-oxo acids formed by the action of GTK on L-cystine, L-cystathionine, and L-thialysine and their metabolic products are present in brain (reviewed in [31]). Results showing that GTK acts naturally on sulfur-containing amino acids in brain in vivo, despite high levels of endogenous levels of glutamine (∼5 mM), suggest that the sulfur-containing amino acids found in the allium extracts might also undergo GTK-catalyzed transamination in vivo.

2-Oxo acids have been suggested to act as anti-oxidants. Given that the sulfur in the allium-derived L-cysteine *S*-conjugates is in a low oxidation state, the 2-oxo acids

produced from these amino acids might act as good antioxidants by a double mechanism involving both the 2-oxo moiety and the sulfur atom. Moreover, the potential exists for interactions of the sulfur-containing 2-oxo acids with enzymes that normally utilize 2-oxo acids as substrates, such as aminotransferases, 2-hydroxy acid dehydrogenases and 2-oxo acid dehydrogenase complexes.

Our findings that SAC is an aminotransferase substrate of purified rat kidney GTK, but not a β-lyase substrate, is in accord with those of Commandeur et al. [33], who demonstrated that *S*-methyl, *S*-ethyl, *S*-allyl- and various *S*-aryl-L-cysteine *S*-conjugates are not detectable (or barely detectable) β-lyase substrates, but are moderately good aminotransferase substrates of rat kidney GTK. Interestingly, however, the selenocysteine-*Se*-conjugate analogues of these cysteine *S*-conjugates are excellent aminotransferase and β-lyase substrates. The selenocysteine Se-conjugates are 5–10-fold better aminotransferase substrates than the sulfur-containing counterparts. The greater reactivity of the selenocysteine Se-conjugates may be due to a weaker C–Se bond than a C–S bond and/or to more facile abstraction of the β proton [33]. These findings may be of relevance to garlic, because this plant is seleniferous and synthesizes selenoalkyl derivatives homologous to the mercaptoalkyl derivatives, e.g. γ-glutamyl-*Se*-methylselenocysteine and selenomethionine, when grown in soils enriched with selenium [45]. Studies by Rooseboom et al. [46] suggest that the selenium homologues of the cysteine *S*-conjugates would be effective β-lyase substrates of GTK (and possibly other cysteine *S*-conjugate β-lyases, including γ-cystathionase). Thus, selenocysteine Se-conjugates containing moieties such as RSe-, RSSe-, and RSeSe- should undergo β-elimination reactions more readily in vivo than cysteine *S*-conjugates containing RS- and RSS- moieties. Although the chemical properties of sulfur resemble those of selenium, organoselenium compounds encompass much greater oxidoreductive potentials [47]. Selenium compounds can cause oxidation of sulfhydryl coordination moieties in many signaling proteins and transcription factors and thereby affect the intracellular sulfhydryl redox potential [48]. Future studies must consider important interactions between sulfhydryl domains within redox sensitive proteins and diet-derived cysteine *S*-conjugates and organoselenium analogues [48].

4.4. Possible biological effects associated with the β-lyase reaction on allium-derived cysteine S-conjugates

Several enzymes are inactivated by L-cystine in crude liver homogenates [49], including tyrosine aminotransferase [50,51] due to thiol modification as a result, at least in part, of the action of γ-cystathionase on L-cystine [51]. The products of the β-lyase reaction on L-cystine are initially pyruvate, ammonia and L-thiocysteine (L-cysteine persulfide) (Eq. (2)). However, interaction of L-thiocysteine with unreacted L-cystine can generate L-cysteine and L-thiocystine (cystine persulfide) (Eq. (3)) [52]. At neutral pH, L-thiocystine slowly breaks down to L-cystine and elemental sulfur (S^0) (Eq. (4)) [53]. Elemental sulfur can then form persulfides with cysteine residues, thereby inactivating tyrosine aminotransferase and other susceptible enzymes [51]. L-Thiocystine is a substrate of rhodanese, which can transfer S^0 (sulfane sulfur) to a suitable co-substrate such as cyanide, which generates the less toxic thiocyanate (Eq. (5)) [53]. The involvement of γ-cystathionase in cyanide detoxification is suggested by the finding that cyanide is more toxic in rats treated with propargylglycine [53].

Generally, interactions involving GSH and cysteine are slow at acid or neutral pH values [54]. Therefore, it is probable that mercapto-L-cysteine *S*-conjugates can enter the bloodstream intact after ingestion. This raises some intriguing biochemical possibilities. β-Lyase reactions involving the allium-derived mercapto-L-cysteine *S*-conjugates should theoretically generate reactive persulfide (hydrodisulfide) species (RSSH), in a similar fashion to the generation of persulfide from cystine.

$$\mathrm{H_3^+N(^-O_2C)CHCH_2SSCH_2CH(CO_2^-)NH_3^+ + H_2O} \rightarrow \mathrm{CH_3C(O)CO_2^- + NH_4^+ + HSSCH_2CH(CO_2^-)NH_3^+} \quad (2)$$

$$\mathrm{H_3^+N(^-O_2C)CHCH_2SSCH_2CH(CO_2^-)NH_3^+ + HSSCH_2CH(CO_2^-)NH_3^+} \rightarrow \mathrm{H_3^+N(^-O_2C)CHCH_2SSSCH_2CH(CO_2^-)NH_3^+ + HSCH_2CH(CO_2^-)NH_3^+} \quad (3)$$

$$\mathrm{H_3^+N(^-O_2C)CHCH_2SSSCH_2CH(CO_2^-)NH_3^+} \rightarrow \mathrm{H_3^+N(^-O_2C)CHCH_2SSCH_2CH(CO_2^-)NH_3^+ + S^0} \quad (4)$$

$$\mathrm{H_3^+N(^-O_2C)CHCH_2SSSCH_2CH(CO_2^-)NH_3^+ + CN^-} \rightarrow \mathrm{H_3^+N(^-O_2C)CHCH_2SSCH_2CH(CO_2^-)NH_3^+ + SCN^-} \quad (5)$$

The present finding that SAMC, *S*-propyl-L-cysteine and *S*-penta-1,3-dienylmercapto-L-cysteine are β-lyase substrates of γ-cystathionase in rat liver homogenates suggests that the eliminated persulfide fragment may be a source of sulfane sulfur through interactions with L-cystine or GSSG.

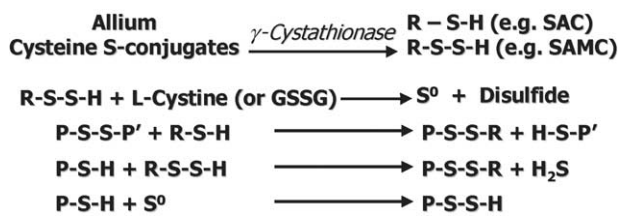

Fig. 3. Possible mechanisms whereby proteins are modified via products generated from the γ-cystathionase reaction. P and P′ represent protein backbones and R represents *S*-alkane and *S*-alkene moieties.

This sulfane sulfur could exert biological effects through addition to cysteine domains on redox sensitive proteins and through detoxification mechanisms involving rhodanese. Possible mechanisms whereby the eliminated sulfur-containing fragments interact with protein sulfhydryls are summarized in Fig. 3.

4.5. Implications for anti-cancer therapy

Numerous studies have shown that several garlic-derived cysteinyl *S*-conjugates have anti-proliferative and pro-apoptotic effects against a number of hormone-sensitive and -refractory human tumor cell lines and inhibit growth of transplantable tumors in experimental animals. As reviewed by Toohey [55,56] and by Iciek and Włodek [57], these effects may be mediated by sulfane sulfur-containing compounds or sulfane progenitors. Toohey [55] suggested that proliferation of malignant cells may be related to a paucity of sulfane sulfur in these cells and to uncontrolled activity of enzymes normally inactivated by sulfane sulfur. Iciek and Włodek [57] strongly endorse the idea that the anti-cancer effects of garlic compounds are due in part to sulfane sulfur production. If this hypothesis is correct the anti-cancer effects of the allium-derived cysteine *S*-conjugates may be due at least in part to their ability to act as progenitors of sulfane sulfur via interactions involving γ-cystathionase.

Flow cytometric analyses of DNA indicate that garlic-derived L-cysteine *S*-conjugates can prevent a variety of tumor cell lines from progressing through G_1/S phase while others are blocked in G_2/M. Studies on cell cycle progression suggest that allylsulfide derivatives can modify redox sensitive signal transduction pathways that lead to expression of nuclear transcription factors. For example, a water-soluble allylsulfide derivative blocks activation of nuclear factor kappa B (NFκB) in Jurkat T cells while one lipid-soluble allylpolysulfide compound induces apoptosis in human promyeloleukemic cells (e.g. reviewed in ref. [7]). Because of the interaction of garlic-derived *S*-allylsulfides with γ-cystathionase (and in one case GTK) to produce reactive sulfhydryl and/or sulfane sulfur intermediates, the present study strongly suggests that the chemopreventive activity of these dietary factors may be due in part to their ability to modify intracellular redox potentials, to generate sulfane sulfur and/or to interact with thiols associated with cysteine moieties in regulatory or catalytic signal proteins.

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