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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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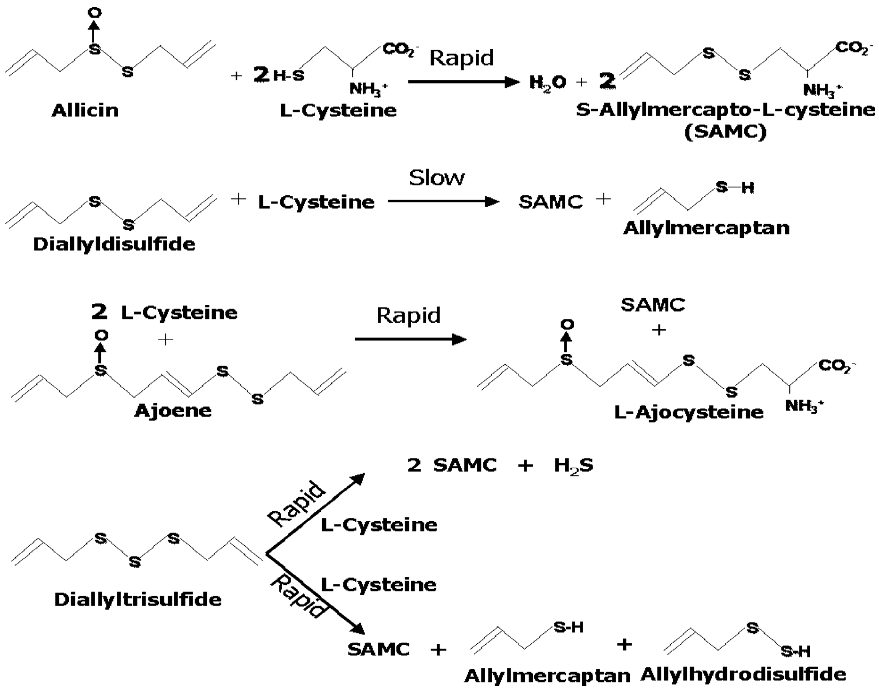

Fig. 1. Formation of *S*-allyl-L-mercaptocysteine (SAMC) from allium-derived polysulfides. The polysulfides are derived from L-alliin [CH_2=$CHCH_2S(O)CH_2CH(N^+H_3)CO_2^-$] in crushed or minced garlic preparations. The reactions with L-cysteine can take place within the lumen of the small intestine 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