REVIEW ARTICLE

Allyl sulfur compounds and cellular detoxification system: effects and perspectives in cancer therapy

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Abstract Natural organosulfur compounds (OSCs) have been shown to have chemopreventive effects and to suppress the proliferation of tumor cells in vitro through the induction of apoptosis. The biochemical mechanisms underlying the antitumorigenic and anti-proliferative effects of garlic-derived OSCs are not fully understood. Several modes of action of these compounds have been proposed, and it seems likely that the rate of clearance of allyl sulfur groups from cells is a determinant of the overall response. The aim of this review is to focus attention on the effects of natural allyl sulfur compounds on the cell detoxification system in normal and tumor cells. It has been already reported that several natural allyl sulfur compounds induce chemopreventive effects by affecting xenobiotic metabolizing enzymes and inducing their down-activation. Moreover, different effects of water- and oil-soluble allyl sulfur compounds on enzymes involved in the detoxification system of rat tissues have been observed. A direct interaction of the garlic allyl sulfur compounds with proteins involved in the detoxification system was studied in order to support the hypothesis that proteins possessing reactive thiol groups and that are involved in the detoxification system and in the cellular redox homeostasis, are likely the preferential targets of these compounds. The biochemical transformation of the OSCs in the cell and their adducts with thiol functional groups of these proteins, could be considered relevant events to uncover the anticancer properties of the allyl sulfur compounds. Although additional studies, using proteomic approaches and transgenic models, are needed to identify the molecular targets and modes of action of these natural compounds, the allyl sulfur compounds can represent potential ideal agents in anticancer therapy, either alone or in association with other antitumor drugs.

Abbreviations

2-PTS	Sodium-2-propenyl-thiosulfate
AMS	Allyl methyl sulfide
BBM	Brush-border membranes
CAR	Constitutive androstane receptor
GS-DNB	Glutathione-2,4-dinitrobenzene conjugate
DAS	Diallyl sulfide
DADS	Diallyl disulfide
DASO	Diallyl sulfoxide
DASO$_2$	Diallyl sulfone
DATS	Diallyl trisulfide
DCF-DA	2′,7′-Dichlorodihydrofluorescein diacetate
DMDS	Dimethyl disulfide
DMH	Dimethyl-hydrazine
DTT	Dithiothreitol
GSH	Glutathione
GST	Glutathione *S*-transferase
HO 1	Heme oxygenase 1
MDR	Multidrug resistance
MRP	Multidrug resistance protein
MST	3-Mercaptopyruvate sulfurtransferase
NQO1	NAD(P)H: quinone oxidoreductase 1
Nrf2	NF-E2-related factor-2
Oatp4	Organic anion transporting polypeptide 4

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OSCs	Organosulfur compounds
P450 s	Cytochrome P450 enzymes
P-gp	P-glycoprotein
ROS	Reactive oxygen species
SAC	S-allylcysteine
SAMC	S-allylmercaptocysteine
Trd	Thioredoxin reductase
Trx	Thioredoxin
TST	Thiosulfate sulfurtransferase
UGT	UDP-glucuronosyl transferase

Introduction

The medicinal properties of the *Allium sativum*, known since the Egyptian age, have been attributed to OSCs present both in the oil- and water-soluble fractions. Epidemiologic and preclinical studies support the likelihood of the garlic as a chemopreventive and anticarcinogenic agent. Several lines of evidence point to allyl sulfur compounds as potentially important antitumorigenic agents (Dirsch et al. 1998; Knowles and Milner 1998; Lea 1996; Lea et al. 1999; Li et al. 1995; Pinto et al. 1997, 2001; Sakamoto et al. 1997; Scharfenberg et al. 1990, 1994; Sigounas et al. 1997; Sundaram and Milner 1993, 1996; Takeyama et al. 1993; Welch et al. 1992; Nian et al. 2008). Toohey suggested that the malignant proliferation of cells may be related to a deficiency of sulfane sulfur. In fact, the observed defective sulfur metabolism in cancer cells and the anti-cancer effects in vivo of sulfane sulfur compounds may be due to the uncontrolled activity of a set of enzymes normally inactivated by sulfane sulfur (Toohey 1989). Cutting and crushing of garlic cloves induces the release of the vacuolar enzyme alliinase (alliin lyase EC. 4.4.1.4), which quickly transforms alliin into allicin via reactive sulfenic acids intermediates. Allicin is easily transformed into oil-soluble polysulfides, mostly diallyl disulfide (DADS), also into diallyl sulfide (DAS), diallyl trisulfide (DATS) and diallyl tetrasulfide. The reactions of allicin can occur both exogenously and endogenously with -SH groups yielding mono-, di- and triallylsulfinyl analogs as well as S-allylcysteine (SAC) or S-allylmercaptocysteine (SAMC), which are water-soluble compounds (Rabinkov et al. 2000; Rosen et al. 2001). The protective effect of these compounds may arise from several mechanisms and it is likely that more cellular events are occurring simultaneously and account for the widespread protection observed experimentally after garlic supplementation. The chemopreventive action of OSCs opens significant questions concerning their effects on the proteins involved in the detoxification process. Although the mechanism of anticancer efficacy of

garlic is not still clear, it has been observed that garlic compounds affect xenobiotic metabolizing enzymes in such a way that carcinogens are less activated or excreted rapidly and the DNA damage is circumvented. Moreover, the direct binding of toxins through the sulfhydryl group of OSCs has also been proposed (Shenoy and Choughuley 1992). Biotransformation of xenobiotics is important to protect all living organisms from environmental toxic insult. It consists of xenobiotic-metabolizing enzymes that are usually classified as phase I and phase II enzymes in mammalian systems.

Effects of garlic OSCs on phase I and phase II detoxification enzymes

Cytochrome P450 enzymes (P450s), the most important phase I enzymes, catalyze the microsomal biotransformation of many xenobiotics and endogenous compounds by hydroxylation, oxidation, or hydrolysis yielding modified derivatives (Buck 1997; Meyer and Rodvold 1996). Many Cytochrome P450 genes are selectively enhanced or suppressed by a variety of chemicals, including numerous garlic components (Reicks and Crankshaw 1996; Yang et al. 1994). For instance, DAS, DADS, and allyl methyl sulfide (AMS) suppress hepatic P450 2E1 protein expression and N-nitrosodimethylamine demethylase activity (Brady et al. 1991a, b; Haber et al. 1995a, b; Davenport and Wargovich 2005; Wu et al. 2002), whereas rat hepatic P450 2B1 protein content was induced (Haber et al. 1995a, b; Pan et al. 1993). P450 2E1 mediates the oxidation of DAS to diallyl sulfoxide (DASO) and subsequently to diallyl sulfone ($DASO_2$) (Brady et al. 1991a, b; Jin and Baillie 1997), and it is likely that P450 2E1-mediated oxidation of the terminal double bonds of $DASO_2$ leads to the autocatalytic destruction of the enzyme (Jin and Baillie 1997). Moreover, the formation of other electrophilic species such as allyl sulfenic acid or acrolein, a highly reactive aldehyde, which could inactivate the cytocrome P450 by alkylation of a critical nucleophilic residue, also may play an important role in the inhibition of P450 2E1-mediated bioactivation of carcinogenic agents in vivo (Gurtoo et al. 1981; Jin and Baillie 1997).

In vivo effects of some OSCs on the modification of some P450 isoenzymes and the activation of various carcinogens have been tested, showing that both dipropyl sulfide and disulfide strongly enhance pentoxyresorufin O-dealkylase activity, while slightly increasing ethoxyresorufin O-deethylase and methoxyresorufin O-demethylase activities of P450 1A family (Guyonnet et al. 2000). The stabilization and induction of these enzymes may prevent the metabolic activation of procarcinogens, increase the clearance rate of toxic metabolites, and become relevant in the anticarcinogenic properties associated with garlic and

allyl sulfur components. The use of natural agents, mainly of dietary origin, early in the disease process may retard or prevent the appearance of resistant neoplastic clones.

The activation of the detoxification pathways by the induction of phase II enzymes such as glutathione S-transferase (GST), epoxide hydrolase, quinone reductase, and UDP-glucuronosyl transferase (UGT), which accelerate the clearance rate of toxic compounds, is also one of the main mechanisms proposed for the chemopreventive effects of OSCs (Bose et al. 2002; Andorfer et al. 2004). Recently, it has been reported that the regulation of the drug-metabolizing enzymes by DAS, DADS, and DATS is obtained by activation of two transcription factors, constitutive androstane receptor (CAR) and NF-E2-related factor-2 (Nrf2) (Fisher et al. 2007). CAR plays a key role in the control of drug metabolism by mediating the induction of many phase I and II drug-metabolizing enzymes (such as P4502B, P4502C, P4503A, UGT1A1, and GSTα1), as well as drug transporters, including Mrp2 and organic anion transporting polypeptide 4 (Oatp4) (Huang et al. 2003; Arnold et al. 2004).

Diallyl sulfur compounds from garlic and onion have been reported to increase also the activity of the phase II detoxification enzymes NAD(P)H: quinone oxidoreductase 1 (NQO1) and GSTs in a variety of rat tissues (Guyonnet et al. 1999; Munday and Munday 1999; Singh et al. 1998). The administration of DAS, DADS, and DATS in human hepatoma HepG2 cells induces changes in the transcriptional levels of NQO1 and heme oxygenase 1 (HO 1) genes (Chen et al. 2004; Fukao et al. 2004). An up-regulation of NQO1 gene expression is induced by treatment with all three tested compounds. In particular, DATS elicited the strongest inductive effect among them. Moreover, HO 1 gene expression is also increased by treatments with DADS and DATS, but not DAS (Chen et al. 2004). DATS, possessing sulfane sulfur (–S–*S–S) in its structure, is more active than the other sulfur compounds in the induction of detoxifying enzymes as demonstrated by Chen and Wu (Chen et al. 2004 and 2002).

In the past decade, special emphasis has been placed on the study of the effects of the garlic OSCs on the GST enzymes. GSTs are detoxification enzymes, which have been recently considered as either phase I or phase II enzymes that catalyze the conjugation of a wide variety of electrophile agents and carcinogens with glutathione (GSH) (Hayes and Pulford 1995). This reaction is the first step in the formation of mercapturic acids, a pathway resulting mostly in the elimination of potentially toxic compounds (Boyer and Kenney 1985; Mannervik et al. 1985). GSTs are also involved in the metabolism of several types of anticancer drugs (Tew 1994) and are overexpressed in many human persistent tumors (Tsuchida and Sato 1992). Chemopreventive effects of garlic constituents are associated with increased levels of GSH and with both significant increase of GST activity in rats treated with DADS (Demeule et al.

2004) and increased levels of GSTα and GSTπ in kidney (Dwivedi et al. 1996; Guyonnet et al. 1999). It has been also observed that the stimulation of hepatic GST by OSCs may require quite a long lag time to evidence an effect (Sumiyoshi and Wargovich 1990; Sparnins et al. 1986, 1988). Although not all GST isozymes are influenced equally and the GST response to OSCs shows organo-specificity, the up-regulation of the GSTπ induced by OSCs may represent a particularly important event in the anticarcinogenic properties associated with garlic (Hu and Singh 1997; Tsai et al. 2007). Moreover, a significant decrease in GST activity was also observed in the hepatocytes after treatment with high concentration of DADS (2 mM) (Sheen et al. 1999).

It is also notable that although DADS significantly increased GST activity in rats, the SAC was not able to affect it (Demeule et al. 2004). By contrast, Sumiyoshi and Wargovich (1990) observed that the oral administration of SAC led to prevention of dimethyl-hydrazine (DMH)-induced nuclear aberrations in mouse colon and that pre-treatment with SAC significantly inhibited the development of DMH-induced tumors in long-term tumorigenesis tests in mouse. SAC significantly increases GST activity in liver and colon, suggesting that the increased GST activity may be responsible for chemopreventive activities of SAC. In agreement with these studies, Hatono et al. (1996) showed an increase of GST activity in liver, intestine, and colon in rat after SAC treatment. In particular, GST activity increased in the colonic mucosa, where carcinomas are ultimately induced by injection of DMH, and the hepatic GSTα and GSTμ, but not GSTπ increased significantly after oral administration of SAC.

Water-soluble OSCs seem to have a different effect with respect to the oil-soluble compounds and OSCs containing allyl groups, which were more significantly stimulatory for GST activity. The allium-derived compounds with mercapto-moiety, like SAMC, have antiproliferative effects through the direct binding of their metabolite to redox-sensitive sites on enzymes or transcription factors inducing the blockage of cellular cycle and leading to the activation of pro-apoptotic-signalling pathway (Shirin et al. 2001; Xiao et al. 2003; Cooper and Pinto 2005). At the moment, there is no clear evidence on the direct interaction of garlic allyl sulfur compounds with the GSTs. We have investigated the effects of the allyl-thiosulfate (2-PTS), which is a garlic-derived water-soluble OSC, on the GST activity and expression both in vitro and in the human T lymphoblastoid cell line, HuT 78.

2-PTS interaction with GST in vitro

The incubation of GST protein, obtained as described by Battistoni et al. (1995), in presence of 2-PTS induces a loss of about 90% of their activity (Fig. 1a) due to covalent modifications of the protein by 2-PTS interaction. In fact, the GST

activity was restored after incubation with a low concentration of thioredoxin (Fig. 1b), while the dialysis or incubation of the mixture at different concentrations of GSH until 10 mM did not lead to the recovery of the activity (data not shown).

In the Fig. 2, the UV difference spectra of the GST untreated/treated with 2-PTS in presence of bromopyruvate are shown. The use of bromopyruvate as an alkylating reagent of GSTπ has been optimized by Lo Bello et al. (1993, 1995) to identify the presence of thiol protein groups. This haloacid reacts quickly with thiols in a quasi-stoichiometric amount; in addition, bromide ion has a negligible absorbance ($\varepsilon = 300$ M^{-1}cm^{-1} at 220 nm) between pH 4.0 and 10.0. The spectrum of the GSTπ1-PS lacks the negative band at 225 nm (Fig.2b) due to the thiol group of the Cys 47 residue (Lo Bello et al. 1993, 1995). This band is visible in Fig. 2a where the GSTπ1–1 was subjected to the same treatment but in absence of 2-PTS (Fig. 2a). LC–(ESI)MS analyses of the protein before and after the treatment with 2-PTS indicate the presence of
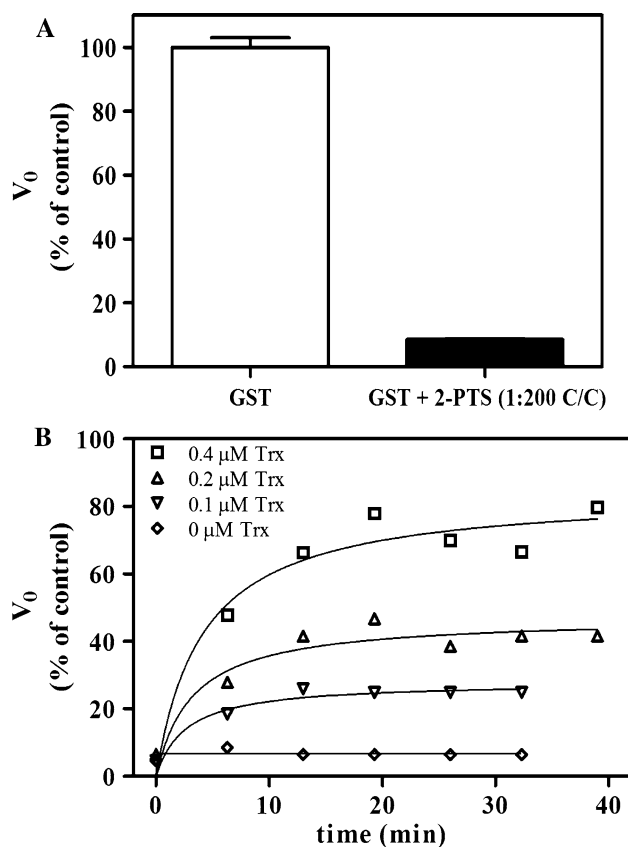

Fig. 1 GST inhibition by 2-PTS a GST was incubated at a molar ratio GST/2-PTS 1:200 c/c in 50 mM phosphate buffer, 7.4 pH, for 2 h at 23°C; b recovery of activity of the GSTπ1-PS form in the presence of different concentrations of thioredoxin (0, 0.1, 0.2, 0.4 μM) at 23°C. The GST activity was evaluated by formation of GS-DNB conjugate (GST assay kit, CS0410-Sigma–Aldrich) (Habig et al. 1974; Mannervik and Danielson 1988; Wilce and Parker 1994) and expressed as initial rate (V_0)
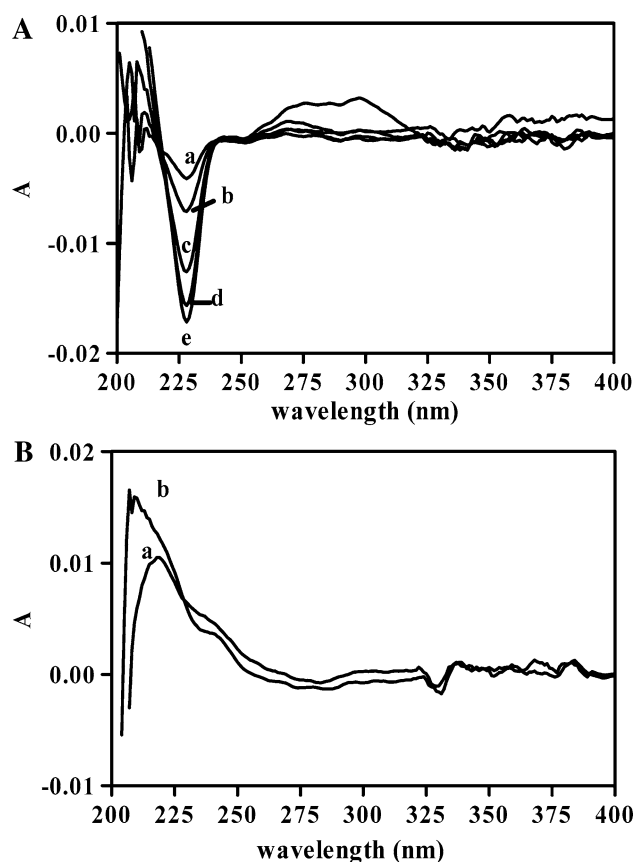

Fig. 2 UV difference spectra of a GSTπ1–1 (2.2 μM) recorded at different times 1 a; 10 b; 20 c; 30 d; 40 e min and b GSTπ1- PS (2.2 μM) recorded at 2 a and 90 b min in 0.1 M phosphate buffer, pH 7.0 in the presence of bromopyruvate (17.6 μM)

three forms of GST with thioallyl groups (23432.9 ± 2.9, 23505.4 ± 3.89 and 23579.6 ± 2.51 m/z) corresponding to GST π1–1 with one, two, and three thioallyl groups, respectively, and the latter was the dominant form present in solution (data not shown). These data, together with the decrease in activity, suggest that the GSTπ1-PS form was thioalkenylated at level of the Cys47, Cys101, and Cys169 residues. In particular, Cys169 is located in the second domain and far from the active site, but may still influence the enzymatic activity; the alkylation of this residue has also been previously observed (Phillips and Mantle 1993). A limited trypsin digestion of the GSTπ1-PS form was also performed to investigate changes in flexibility of the modified enzyme by 2-PTS and to compare it with the untreated GST. In fact, limited proteolysis of globular proteins, generally, occurs at sites, which are the most flexible regions of the polypeptide chain within a domain or at the flexible hinges between domains.

GST appears quite resistant to limited proteolysis, and it is only partially proteolyzed by trypsin while the majority of the protein remained intact after 1 h of incubation (Fig. 3a). By contrast, the GSTπ1-PS form showed a higher

sensitivity to proteolysis than the native form (as showed in Fig. 3b) and a rapid digestion was observed. These data suggest that GSTπ1-PS is more flexible than GSTπ1–1 probably due to conformational changes in the protein with a large increase of regions with local flexibility.

Effects of 2-PTS on GSTπ expression and activity in HuT 78 cells

In a previous work we have observed that 2-PTS was able to induce a typical dose-dependent inhibition of cell growth of the HuT 78 cells, and that cell viability was reduced significantly upon a 24-h exposure to 0.5 mM 2-PTS. This reduced growth rate was related to a blockage in the G$_2$/M phase of the cell cycle (Sabelli et al. 2008). The effects of 2-PTS on GSTπ enzyme expression and GST activity in HuT 78 cells have been analyzed. Densitometry measurements of Western blots of the HuT 78 lysates after 24- and 48 h of treatment with 0.5 mM 2-PTS, corrected for Actin expression, show that no significant variation of the GSTπ expression with respect to the control was induced by treatment with 2-PTS (data not shown). The kinetic experiments show only a small increase of the total GST activity after 24 h of treatment of the cells with 0.5 mM 2-PTS (Fig. 4).

The expression of the GSTπ and the GST activity in the HuT 78 cells are not substantially affected by the treatment with 2-PTS as showed in the case of other water-soluble garlic compounds (Hatono et al. 1996). However, it is not possible to exclude a modulation of the expression of other GST isozymes. Moreover, at high concentrations of 2-PTS a possible effect of up-regulation of the expression of GSTs could be balanced by a direct inhibition effect and an increase of the sensitivity to proteolytic degradation. Although the presence in the cell of the molecules involved
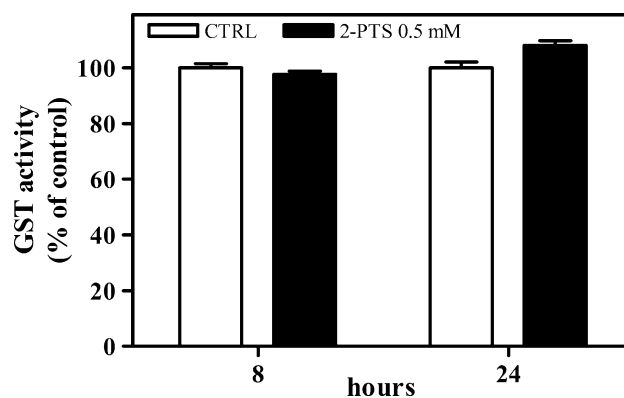
Fig. 4 Effects of 2-PTS on GSTπ activity in HuT 78 cells. GST activity of the HuT 78 cells after 8 and 24 h of treatment with 2-PTS

in maintaining the redox-state of the cell, such as thioredoxin and GSH, can reduce the effects of the allyl sulfur compounds on the redox center of the proteins, the decrease of the availability of the reduced forms of these molecules could induce a redox stress. Taken together, these studies suggest that the antitumor effects of the garlic OSCs can be related to a significant modulation of both phase I and phase II metabolism of xenobiotics (see Table 1), which can directly influence carcinogen activation and/or the apoptosis induction in the tumor cells.

Multidrug resistance protein and P-glycoprotein

Generally, GSH-conjugates are exported from cells by energy-dependent GS-xenobiotic pumps, also known as the Multidrug Resistance Proteins (MRPs), which represent phase III of the detoxification system (Borst et al. 2000). The multidrug resistance (MDR), principally due to the drug efflux proteins P-glycoprotein (P-gp) and Mrp, is one
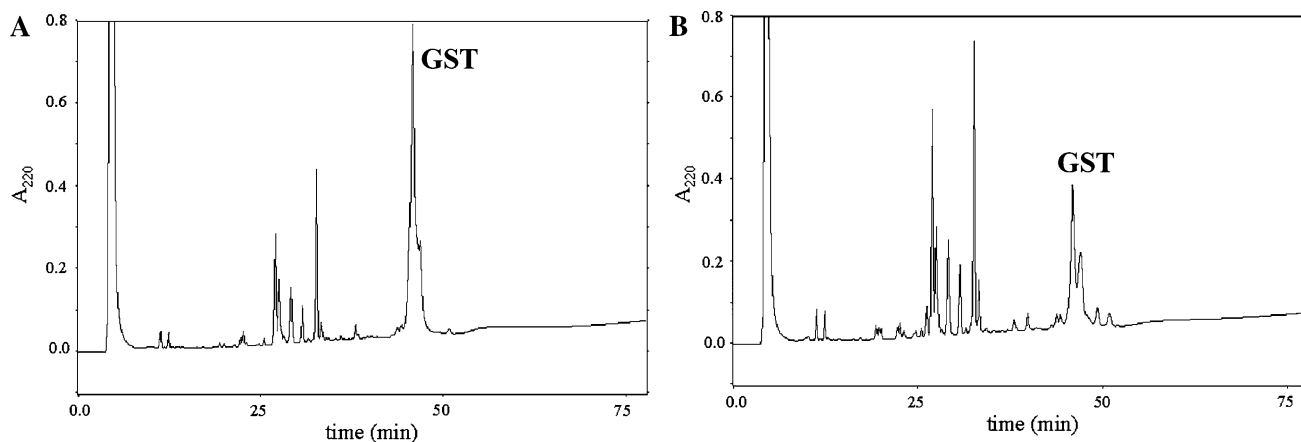
Fig. 3 Limited proteolysis of GST by Trypsin. **a** GST and **b** GSTπ1-PS (1 mg) were subjected to limited-digestion with 1% trypsin (w/w), in 100 mM ammonium bicarbonate buffer, pH. 8.02, for 1 h at 37°C. The reaction was stopped by addition of acetic acid and the samples were subjected to analysis by RP-HPLC, using a solv. B gradient: 0–40 min, 60%; 40–45 min, 60% and 45–70 min, 90% and a Brouwnlee C-18 column (OD-300, 250 × 4.6 mm, 7 μm). 0.1% TFA and 80% CH$_3$CN, 0.1% TFA was used as A and B solvent, respectively

Table 1 Inhibition and induction of the activity and/or expression of the enzymes, involved in the detoxification process, by allyl sulfur compounds

	Inhibition	Induction
DAS	P450 2E1[1-5]	P450 A1,A3[6, 7]
	N-nitrosodimethylamine	P450 2B[3, 8, 9]
	Demethylase[2]	NQO1[10-12]
	P-gp[13]	GSTπ[13],α,μ[14]
DADS	P450 2E1[1-5]	P450 2B[3, 8, 11,]
	GST[15]	NQO1[7, 10, 11]
		HO 1[9, 10]
		GSTα, μ, π[16-18]
		Mrp2[19]
		TST[20]
		MST[21]
DATS		NQO1[10, 11]
		HO 1[9, 10]
		GSTα[21], μ, π[16, 17]
		GSTYb1, Yc[21]
AMS	P450 2E1[2]	
SAC	P-gp[12,19]	GSTα, μ[22, 23]
2-PTS	TST[24]	
	GSTπ	

[1] Brady et al. (1991a); [2]Brady et al. (1991b); [3]Haber et al. (1995a, b); [4]Davenport and Wargovich (2005); [5]Wu et al. (2002); [6]Guyonnet et al. (2000); [7]Le Bon (2003); [8]Pan et al. (1993); [9]Chen et al. (2004); [10]Singh et al. (1998); [11]Fisher et al. (2007); [12]Arora et al. (2004); [13]Hu et al. (1996); [14]Dragnev et al. (1995); [15]Sheen et al. (1999); [16]Dwivedi et al.(1996); [17]Guyonnet et al. (1999); [18]Andorfer et al. (2004); [19]Demeule et al. (2004); [20]Iciek et al. (2005); [21]Wu et al. (2001); [22]Hatono et al. (1996); [23]Sumiyoshi and Wargovich (1990); [24]Sabelli et al. 2008

of the major obstacles to successful cancer chemotherapy. In particular, the expression of the ATP-binding cassette transporter P-gp has been linked to the development of MDR in human cancer, such as leukemias, lymphomas, multiple myeloma, neuroblastoma, and soft tissue sarcoma (Gottesman and Pastan 1993; Malayeri et al.1996). A plethora of agents have been developed to inhibit or modulate the MDR system and to enhance the antitumor activity of anticancer drugs. It has been observed that DADS and SAC modulate the expression of both Mrp2 and P-glycoprotein (P-gp) in rat renal brush-border membranes (BBM) (Demeule et al. 2004). Mrp2 is an ATP-dependent transporter for organic anions, identified in the membrane of hepatocytes and present in the renal BBM and intestine (Evers et al. 1998; Schaub et al. 1997), and contributes to drug resistance by transporting a wide range of glutathione (GSH), glucuronate, and sulfate conjugates out of the cells (Ishikawa and Ali-Osman 1993; Paulusma and Oude Elferink 1997). DADS induces Mrp2 expression and co-treatment with OSC and cisplatin leads to a 30-fold
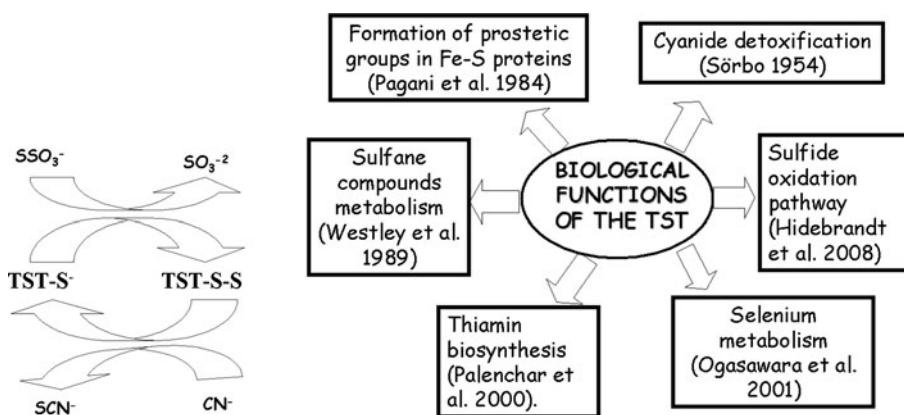
increase of Mrp2 expression. On this basis, it has been suggested that Mrp2 could be involved in the secretion of cisplatin–GSH or DADS–GSH conjugates. Interestingly, the co-treatment with SAC and cisplatin decrease P-gp protein expression (Demeule et al. 2004). The garlic OSCs DADS, SAC, and NAC modulate P-gp mRNA levels in renal cortex only in co-treatment with other antitumor drugs, such as cisplatin. Cisplatin treatment, in fact, induces P-gp expression in renal BBM suggesting that this multidrug transporter may be involved in the renal response to drug cytotoxicity (Demeule et al. 2004). It has been observed that DAS is a selective and highly potent modulator of P-gp-mediated MDR in human K562 leukemic cells and in rodent liver (Arora et al. 2004). The ability of DAS to increase the accumulation of doxorubicin in vinblastine-resistant K562/R cells supports the conclusion that DAS acts by interfering with a process associated with the expression of P-gp inducing a time-dependent reduction in P-gp levels (Arora et al. 2004). However, at the moment the possibility of other mechanisms of action of the DAS cannot be ruled out. OSCs present in a garlic-rich diet might also affect the chemotherapeutic treatments by direct interaction with P-gp; for this reason, further studies are required to clarify the mechanism of allyl sulfur compounds in affecting the MDR.

OSCs in the cyanide detoxification

The cyanide detoxification system in the organisms is principally due to the enzyme rhodanese (thiosulfate: cyanide sulfurtransferase, TST, EC. 2.8.1.1), which is a ubiquitous enzyme known to be responsible for biotransformation of cyanide to thiocyanate (Sörbo 1953; Westley 1973). TST performs a variety of physiological functions starting from the detoxification of cyanide, to the biogenesis of iron-sulfur clusters (Pagani et al. 1984), transport mechanisms of sulfur/selenium in a biological available forms (Ogasawara et al. 2001; Westley and Westley 1989), and sulfide oxidation pathway (Hidebrandt et al. 2008) (Fig. 5). It has also been found that different parts of the digestive system contain higher rhodanese activity than liver (Aminlari and Gilanpour 1991; Aminlari et al. 1997). Although the biological role of this enzyme remains still elusive, the thiosulfate-cyanide sulfurtransferase represents a link between the cyanide detoxification system and OSCs metabolism.

Recent studies show an increase in activity of sulfurtransferases and sulfane sulfur levels in liver induced by DADS treatment accompanied by the changes of the number of Gomori-positive cytoplasmic granulation in the mouse brain (Iciek et al. 2005). However, DADS does not significantly influence the activities of TST in tumor-bearing mice (Iciek et al. 2005). Recently, we have found

that garlic-derived 2-PTS is able to interact with the active site of the rhodanese enzyme inducing thiolation of the catalytic cysteine, forming a characteristic disulfide bond which is not cleavable by a nucleophilic attack of cyanide (Sabelli et al. 2008). A significant reduction of TST activity has been observed during 2-PTS treatments indicating that cyanide detoxification of rhodanese is reduced by the presence of 2-PTS. On the contrary, no change of the rhodanese expression was observed after 8 and 24 h of treatment with 2-PTS. TST could be one of target enzymes of the garlic OSCs and the reduced TST activity could be due to an increase of the sulfur detoxification activity of the enzyme, which directly involves also the thioredoxin system. The ability of 2-PTS to inhibit TST activity, both in vitro and in cell, may be also related with its ability to induce apoptosis of tumor cells by mitochondrial dysfunction. A strict correlation between 2-PTS apoptotic effects and oxidative imbalance has been also observed and can be linked to a reduction in the activity of oxygen radical-detoxification of the rhodanese-thioredoxin system in cell (Sabelli et al. 2008). At the moment, little information is available on the effects of other ally sulfur compounds on rhodanese expression and activity in tumor and non-tumor cells. However, the ability of this garlic compound to thiolate the internal Cys of the active site of the rhodanese is an important observation to be considered for understanding the mechanism of action also of other allyl sulfur compounds. Moreover, other studies are necessary to investigate the role of this mitochondrial enzyme in cancer suppression by allyl sulfur compounds and in chemoprevention.

Conclusions

An important issue in cancer treatment is therapeutic selectivity. Not all cells are equally susceptible to the deleterious effects of the garlic sulfur compounds and, in particular, neoplastic cells tend to be more susceptible.

This has suggested that uncontrolled proliferation of the tumor cells may be also related to an incorrect functionality of the enzymes involved into a sulfane sulfur metabolism and in the detoxification system. Thus, this evidence places the natural OSCs as potential ideal agents in anticancer therapy. Active sulfur metabolizing enzymatic system could be beneficial to the cell leading to a low concentration of reactive and toxic sulfur species, that in high levels could induce apoptosis as observed in neoplastic cells. The natural allyl sulfur compounds could have a similar behavior to that observed for other natural antioxidant agents. In fact, low levels of natural antioxidant compounds induce an increased cell survival following DNA damage; on the contrary, a high concentration caused the opposite effect (Howitz et al. 2003). Therefore, a relevant point to be investigated more deeply is the dose-dependence of the effects due to the allyl sulfur compounds and, in particular, major attention should be applied to the estimation of the concentrations that can be attained by normal dietary intake in order to compare them with the data obtained from in vitro studies. The chemopreventive effects of the garlic on the mammals and the induction of the apoptosis in tumor cells could be explained both by a different sensitivity of the cancer cells to these compounds and by the different concentration of the reactive species used in these studies. Albeit the cancer suppression by induction of programmed cell death after treatment with allyl sulfur compounds has been related to several epigenetic changes, a direct inhibitory action of allyl sulfur compounds from garlic on enzymes involved in the detoxification system and in the control of the redox state of the cell (e.g. GST, mitochondrial TST, etc.) may be considered as a relevant event of their mechanism of action. Several proteins involved in essential cellular processes are, in fact, characterized by the presence of reactive thiol groups, and the anticancer properties of the allyl sulfur compounds may be related to both their biochemical transformation in the cell and their reactivity with thiol groups on redox-sensitive and detoxification proteins.

Thioalkenylation of these reactive centers, that enhance the protein degradation, may be an important mechanism in apoptosis induction by several garlic allyl sulfur compounds.

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