Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 569 (2007) 1 – 7
www.elsevier.com/locate/ejphar

Selective effects of diallyl disulfide, a sulfane sulfur precursor, in the liver and Ehrlich ascites tumor cells

Małgorzata Iciek, Joanna Marcinek, Urszula Mleczko, Lidia Włodek *

Chair of Medical Biochemistry, Collegium Medicum, Jagiellonian University, Kopernika 7, PL 31-034 Kraków, Poland

Received 10 November 2006; received in revised form 6 March 2007; accepted 26 April 2007
Available online 22 May 2007

Abstract

The present in vivo studies demonstrated that diallyl disulfide (DADS), occurring in garlic, elevated hepatic sulfane sulfur level and activities of γ-cystathionase and 3-mercaptopyruvate sulfotransferase in healthy mice but did not affect the hepatic glutathione level. DADS efficiently corrected the concentrations of glutathione and sulfane sulfur, and ameliorated γ-cystathionase activity that had been lowered in the livers of Ehrlich ascites tumor-bearing mice. In Ehrlich ascites tumor cells, diallyl disulfide did not alter bound sulfane sulfur level, sulfotransferases activity or glutathione level. These data indicate that this compound is capable of acting efficiently and selectively only in the liver and can be used for hepatoprotection during chemotherapy.

Keywords: Diallyl disulfide; Sulfane sulfur; Sulfotransferases; Glutathione; Ehrlich tumor

1. Introduction

Sulfane sulfur occurs in compounds in the 0 and −1 oxidation state and is always covalently bound to another sulfur atom. Sulfane sulfur is labile and can easily leave structure of compounds. It is exceptionally metabolically active and can be transferred to different acceptors, like sulfates (IV) (SO_3^{2-}), sulfinates (R–SO_2–H) and cyanide (CN^-). Because of the latter acceptor, sulfane sulfur is also called "cyanolysable sulfur". It is capable of reversible covalent modification of protein—SH groups yielding hydropersulfides (R–S–S–H) or trisulfides (R–S–S–S–R), thereby being able to regulate activities of numerous proteins (Iciek and Włodek, 2001; Toohey, 1989).

Sulfane sulfur-containing compounds are formed during anaerobic L-cysteine metabolism (Cooper, 1983) (Scheme 1). This process is catalyzed by γ-cystathionase (CST) and 3-mercaptopyruvate sulfotransferase (MPST). These enzymes together with rhodanese (TST) and albumin participate in sulfane sulfur transport in the form of hydropersulfides and trisulfides to different acceptors (Scheme 1).

Sulfane sulfur-containing compounds not only fulfill a regulatory role (Toohey, 1989) but also exhibit antioxidant properties (Everett, 1995; Everett et al., 1994).

Just trace activity of γ-cystathionase, 3-mercaptopyruvate sulfotransferase and rhodanese is a hallmark of Ehrlich ascites tumor cells (Włodek et al., 1993). This indicates that neoplastic transformation deprives the cells of sulfane sulfur and its regulatory role. For this reason, Toohey (1989) has suggested that uncontrolled proliferation of tumor cells can result form sulfane sulfur deficit.

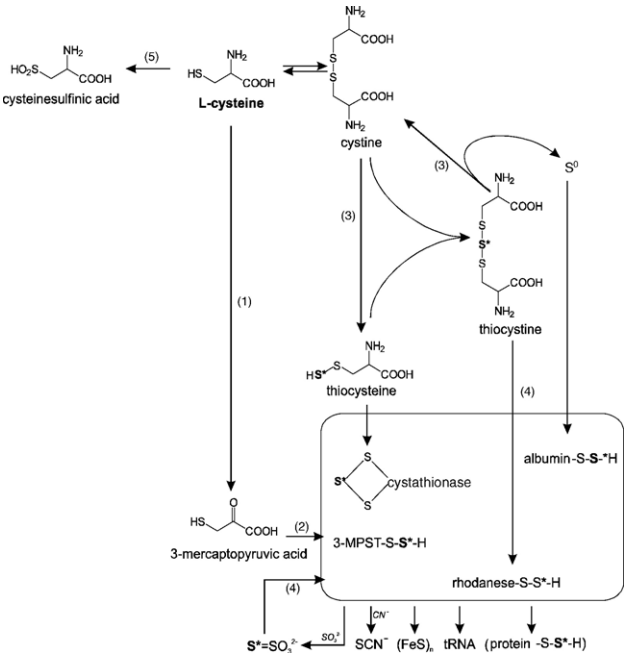
Studies into biological role of sulfane sulfur-containing compounds are hindered by exceptional reactivity and instability of these structures. Hence, the search for exogenous precursors of sulfane sulfur is of fundamental significance. They can be used as a cellular source of sulfane sulfur to facilitate examination of its biological function (Iciek and Włodek, 2001; Toohey, 1986, 1989).

Diallyl disulfide (DADS) originating from garlic belongs to potential sulfane sulfur precursors. It is transformed in tautomerization reaction into thiosulfoxide which is an isomer bearing a labile and reactive sulfur (Scheme 2).

We have chosen diallyl disulfide as the sulfane sulfur precursor on the basis of earlier observations indicating its stronger biological action than isopropyl disulfide or diallyl sulfide

* Corresponding author. Tel.: +48 12 422 74 00; fax: +48 12 422 32 72.
E-mail address: mbwlodek@cyf-kr.edu.pl (L. Włodek).

0014-2999/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2007.04.055



Scheme 1. Cysteine metabolism.

(DAS) (Sundaram and Milner, 1996). Tautomerization reaction can occur if a molecule contains both disulfide bond and unsaturated allyl group. Therefore, stronger biological activity of DADS can result from its tautomerization to thiosulfoxide, thus, it can depend on the formation of labile sulfane sulfur in the molecule (Scheme 2).

Diallyl disulfide (DADS) is the main sulfur-containing component of oil-soluble fraction of garlic extract (Dausch and Nixon, 1990). Undamaged garlic bulbs contain odorless alliin (S-allylcysteine sulfoxide). Tissue damage (crushing, grinding, cutting) induces the release of the enzyme, allinase, which transforms alliin into allicin, the compound with characteristic pungent odor (Amagase, 2006; Herman-Antosiewicz and Singh, 2004). Allicin (allyl-2-propenyl thiosulfinate) is an unstable compound, which can be a source of different sulfur products, like diallyl disulfide (DADS), diallyl sulfide (DAS), or diallyl trisulfide (DATS) (Scheme 3).

The main aim of the studies presented in this paper was to examine whether a potential sulfane sulfur precursor, like DADS, can be used in vivo as a source of sulfane sulfur and whether it can influence activity of enzymes involved in its formation and transfer. In addition, we aimed to investigate whether DADS can affect the level of glutathione and thus, if it can change cellular antioxidant potential. The studies were conducted on livers of healthy and EAT-bearing mice and on Ehrlich ascites tumor cells.

2. Materials and methods

2.1. Animals

Female albino Swiss mice, weighing approximately 20 g were used. In the course of the experiment, the animals were kept under standard laboratory conditions and were fed a standard chow. The mice were randomly divided into 4 groups containing 8 animals each. The experimental animals in the first group were treated ip with DADS at 50 mg per kg of body weight dissolved in corn oil, for 10 days. The control mice were injected with the same volume of the corn oil (group 2). The mice in the third and fourth groups had EAT cells inoculated. On the fifth day, the mice in the third group were injected ip with DADS at 50 mg per kg of body weight for 10 days. The control mice in this group were injected with the same volume of corn oil (group 4).

All mice were sacrificed by cervical dislocation. The Ehrlich ascites tumor cells were collected and then washed three times with cold 0.9% sodium chloride solution, followed by centrifugation at 650 $\times g$ for 5 min. The livers were isolated, placed in liquid nitrogen and stored at −76 °C until used in biochemical experiments.

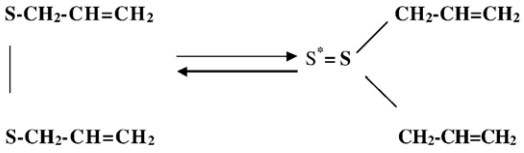
All procedures were approved by the Ethics Committee for the Animal Research in Krakow (nr. 74/OP/2002).

2.2. Chemicals

Diallyl disulfide (DADS), used in in vivo studies was purchased from Fluka Chemie AG Buchs and its purity was 80% (diallyl sulfide devoid of sulfane sulfur constituted 20% of the reagent). The in vitro experiments were performed with DADS from LKT Laboratories (purity >98%). The remaining sulfur compounds: diallyl sulfide (DAS), diallyl trisulfide (DATS) and dipropyl disulfide (DPDS) were also purchased from LKT Laboratories, Inc. (Minnesota, USA). Thiosulfate, formaldehyde, ferric chloride ($FeCl_3$), sodium sulfite were obtained from the Polish Chemical Reagent Company (P.O. Ch, Gliwice, Poland). Dithiothreitol, p-phenylenediamine, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), glutathione reductase, N-ethylmaleimide (NEM), β-nicotinamide adenine dinucleotide reduced form (NADH), β-nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt (NADPH), 3-methyl-2-benzo-thiazolinone hydrazone (MBTH), pyridoxal 5'-phosphate (PLP), homoserine, potasium cyanide (KCN), trichloroacetic acid (TCA) and lactic dehydrogenase (LDH) were provided by Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Preparation of tissue homogenate

The frozen livers were weighed and homogenates were prepared by homogenization of 1 g of the tissue in 4 ml of 0.1 M phosphate buffer, pH 7.4 using IKA-ULTRA-TURRAX T8 homogenizer. Liver homogenates were next used for assay of sulfane sulfur level, activity of sulfotransferases and glutathione level.



Scheme 2. Diallyl disulfide tautomerization into thiosulfoxide containing a sulfane sulfur atom (S*).