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Selenoxides inhibit δ-aminolevulinic acid dehydratase

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Abstract

The effect of two selenides and their selenoxides on δ-aminolevulinic acid dehydratase (δ-ALA-D) from liver of adult rats was investigated. In vivo, selenides can be oxidized to selenoxides by flavin-containing monooxygenases (FMO) and selenoxides can regenerate selenides by thiol oxidation. Phenyl methyl selenide ($PhSeCH_3$) and 1-hexynyl methyl selenide ($C_4H_9C{\equiv}CSeCH_3$) were converted to selenoxides by reaction with H_2O_2. $PhSeCH_3$ and $C_4H_9C{\equiv}CSeCH_3$ had no effect on δ-ALA-D up to 400 μM. Conversely, their selenoxides inhibited δ-ALA-D, and the IC_{50} for enzyme inhibition was about 100 and 70 μM, respectively. Partially purified δ-ALA-D (P_{55}) from swine liver was also inhibited by these selenoxides. The inhibitory action of selenoxides was antagonized by dithiotreitol (DTT). Moreover, δ-ALA-D from a plant source was inhibited by the selenoxides, suggesting a possible involvement of –SH groups in a distinct site of the homologous region implicated in Zn^{2+} binding in mammalian δ-ALA-D. After exposure to $PhSeCH_3$ (500 μmol/kg/day) for 45 or 30 days, the activity of δ-ALA-D from liver of mice decreased to about 50% of the control group. The in vivo inhibitory action of this compound was not antagonized by DTT. $PhSeCH_3$ and $C_4H_9C{\equiv}CSeCH_3$ had no effect on the rate of DTT oxidation, but their selenoxides oxidized DTT. The results of the present study suggest that hepatic δ-ALA-D of rodents is a potential molecular target for selenides as a consequence of their metabolism to selenoxides by FMO.

Keywords: δ-Aminolevulinate dehydratase; Selenoxides; Flavin-containing monooxygenase

1. Introduction

Selenium is an essential trace element that constitutes the active center of glutathione peroxidase (Forstrom et al. 1978; Landestein et al., 1979; Wingler and Brigelius-Flohé, 1999). Moreover, this element is a component of other selenoproteins like 5′-deioidinase (Behne and Kyriakopoulos, 1990) and selenoprotein P (Linder, 1990). In view of the fact that selenium was found to be an essential dietary micronutrient for many mammalian animal species (Oldfield, 1987; Lin-

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der, 1990), dietary selenium supplementation is accepted by the scientific community. On the other hand, it is well known that selenium is highly toxic to several species of mammals like cattle (Hartley and Grant, 1961), sheep (Blodgett and Bevill, 1987), pigs (Penrith, 1995), rats (Anundi et al., 1984) and humans (Cheftel and Truffert, 1972; Frost, 1972; Louria et al., 1972). While the mechanism of selenium toxicity has not been clearly elucidated, Painter (1941) emphasized that the toxicity of inorganic selenium could be related to the oxidation of thiols with the concomitant formation of derivatives of the RSSeSR type (selenotrisulfides). After the theoretical Painter proposal, interaction of thiols with inorganic selenium was confirmed experimentally by various investigators (Tsen and Tappel, 1958; Ganther, 1968). In fact, inorganic selenium compounds (selenite) catalytically increase the oxidation of glutathione and this reaction produces superoxide ($O_2^{\bullet -}$) (Seko et al., 1989).

Flavin-containing monooxygenases (FMO) are flavoproteins of some mammalian tissues that catalyze NADPH- and oxygen-dependent oxidation of a wide variety of xenobiotics (Ziegler, 1988; Cashman, 1995). The catalytic mechanism of the FMO is known in some detail only for the hog liver enzyme (Poulsen and Ziegler, 1979; Beaty and Ballou, 1980), but it is likely that the major steps are essentially the same in other mammals. Kinetic studies have indicated that, in the cell, the enzyme is present largely in the hydroperoxyflavin form and, in principle, compounds that can be oxidized by an organic hydroperoxide are potential substrates for this enzyme (Beaty and Ballou, 1981). In addition, several studies have demonstrated the monooxygenation of dialkyl- and alkylaryl-selenides to their selenoxides by FMO (Bruice, 1982; Goeger and Ganther, 1994). Selenoxides are potent thiol oxidants and can initiate a futile cycle that catalyzes the oxidation of thiols at the expense of NADPH and oxygen (Ziegler et al., 1991).

δ-Aminolevulinate dehydratase (δ-ALA-D) is an essential enzyme in most organisms, catalyzing the condensation of two molecules of 5-aminolevulinic acid (ALA) to form the monopyrrole porphobilinogen (Sassa et al., 1989; Jaffe et al., 1995; Sassa, 1998). δ-ALA-D is a sulfhydryl-containing enzyme and its activity is highly sensitive to the presence of elements such as mercury (Rocha et al., 1993, 1995; Emanuelli et al., 1996, 1998), lead (Rodrigues et al., 1989, 1996; Burns and Godwin, 1991; Flora et al., 1991; Goering, 1993), copper (Nelson et al., 1981), and tin (Chiba and Kikuchi, 1984), which possess high affinity for –SH groups. The enzyme is also inhibited by elements such as aluminum (Schetinger et al., 1999; Vieira et al. 2000), gallium arsenide (Kondo et al., 1996; Flora et al., 1998), and organic selenium and tellurium compounds (Barbosa et al., 1998; Maciel et al., 2000) that oxidize –SH groups or tentatively compete with Zn^{2+}. δ-ALA-D inhibition may impair heme biosynthesis and can result in the accumulation of ALA, which has some pro-oxidant activity (Bechara et al., 1993).

The selenoxides generated by oxidation of selenides via FMO are potent thiol oxidants and, consequently, can oxidize –SH groups of δ-ALA-D, causing enzyme inactivation. In the present study, we reported the in vitro effects of one dialkyl- and one alkylaryl-selenide on the sulfhydryl-containing enzyme δ-ALA-D from a mammalian source. The selenides were reacted with hydroperoxide in order to form their respective selenoxides and the products of these reactions were studied as in vitro inhibitors of δ-ALA-D. The effect of exposure to phenyl methyl selenide on δ-ALA-D activity was also studied in order to identify possible toxic effects of organoseleno compounds towards the sulfhydryl containing enzyme δ-ALA-D.

2. Materials and methods

2.1. Compounds

Dithiothreitol (DTT), 5,5′-dithio-bis(2-nitrobenzoic acid), magnesium chloride, zinc chloride, 5-aminolevulinic acid, Coomassie brilliant blue G, *p*-dimethylaminobenzaldehyde and purified catalase from bovine liver were obtained from Sigma (St. Louis, MO, USA). Hydrogen peroxide (30%), dimethylsulfoxide (DMSO), ethylacetate, potassium permanganate, mono- and

dibasic potassium phosphate, acetic acid, orthophosphoric acid, trichloroacetic acid and sodium chloride were obtained from Merck (Darmstadt, Germany). The selenocompounds phenyl methyl selenide and 1-hexynyl methyl selenide were prepared according to literature methods (Braga et al., 1996). Their structures are presented in Scheme 1.

2.2. Animals

Adult male Wistar rats (aged 2–3 months) and male mice (aged 2–3 months) from our own breeding colony were maintained in a conditioned room (20–25°C) under natural lighting conditions with water and food (Guabi-RS, Brasil) ad libitum. The hogs used for δ-ALA-D purification were obtained from the Sector of Swine Culture of our University.

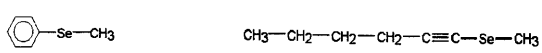
2.3. Tissue preparation

2.3.1. Animals

Rats and mice were killed by decapitation. The liver was quickly removed, placed on ice and homogenized in 7 volumes 150 mM NaCl. The homogenate was centrifuged at $4000 \times g$ at 4°C for 10 min to yield a low-speed supernatant fraction (S_1) that was used for enzyme assay.

2.3.2. Plants

Cucumber (*Pepino oadai*) seeds were germinated for 5–7 days at 25°C. Leaves were homogenized in a medium containing 5 volumes 10 mM Tris–HCl buffer (pH 9.0). The homogenate was then centrifuged as already described for animal tissue and a low-speed supernatant fraction (S_1) obtained was used for enzyme assay.



Phenyl methyl selenide 1-hexynyl methyl selenide

Scheme 1. Structures of selenides.

2.4. Preparation of partially-purified δ-ALA-D

Partially-purified δ-ALA-D (P_{55}) was obtained as previously described (Emanuelli et al., 1998).

2.5. In vivo exposure to phenyl methyl selenide

Mice were injected with phenyl methyl selenide (500 μmol/kg/day) once a day, subcutaneously, five times per week for 30 or 45 days. Subcutaneous route of exposure was selected because phenyl methyl selenide is highly lipophilic. The phenyl methyl selenide was diluted in DMSO and injected at a proportion of 2.5 ml/kg. Control animals were injected with DMSO at a proportion of 2.5ml/kg. The animals were killed 24 h after the last injection and the liver was removed for tissue preparation.

2.6. Enzyme assay

Mammalian δ-ALA-D activity was assayed by the method of Sassa (1982) by measuring the rate of product (porphobilinogen) formation except that 84 mM potassium phosphate buffer (pH 6.4) and 2.5 mM ALA were used. Moreover, for the partially purified δ-ALA-D, the medium contained 100 μM D,L-dithiothreitol. For the plant enzyme, the medium contained 50 mM Tris–HCl buffer (pH 9.0) and 2.5 mM ALA. All experiments were carried out after 10 min of pre-incubation. The reaction was started 10 min after the addition of the enzyme preparation by adding the substrate. Incubations were carried out for 1 h at 39°C for the mammalian enzyme and for 90 min at 35°C for the plant enzyme. The reaction product was determined using modified Ehrlich's reagent at 555 nm, with a molar absorption coefficient of 6.1×10^4 cm^{-1} M^{-1} for the Ehrlich-porphobilinogen salt. The reaction rates were linear with respect to time of incubation and added protein for all experimental conditions.

2.7. Reaction of selenides with hydroperoxide

The selenides (28.5 μM–2.85 mM) dissolved in 10 μl ethanol were mixed with 25 μl H_2O_2 to give a final concentration of 30 mM. The reaction was

carried out for 1 h at 39°C and stopped by adding catalase (200 U). The same tubes were used for the enzyme assay and the final concentrations of the chalcogens ranged from 4 to 400 μM. The absence of H_2O_2 was assessed in parallel tubes by tritiating with potassium permanganate ($KMnO_4$).

2.8. Determination of the rate of DTT oxidation

Reaction of selenides with H_2O_2 were carried out as described in Section 2.7, except that H_2O_2 (125 mM) and selenides (0.7–14 mM) were used, and the final concentrations of chalcogens ranged from 0.1 to 2 mM. After catalase addition, 84 mM potassium phosphate buffer (pH 6.4) was added. DTT (2 mM) oxidation was evaluated by measuring the disappearance of –SH groups by the method of Ellman (1959). Incubation at 39°C was initiated by adding DTT in a total volume of 250 μl. Aliquots of 50 μl were sampled at 0, 30 and 60 min to determine the amount of –SH groups at 412 nm.

2.9. Protein quantification

Protein was measured by the method of Bradford (1976) using bovine serum albumin as standard.

2.10. IC_{50} determination

The IC_{50} for in vitro inhibition of δ-ALA-D was calculated by the method of Dixon and Webb (1964).

2.11. ^1H-NMR studies

The selenides were incubated with H_2O_2 at a ratio 1:10 (mol/mol) at 39°C for 24 h. The organic phase was extracted with ethylacetate and analyzed with a Brucker DPX-200 (200 MH_Z) spectrometer for solution in $CDCl_3$ with tetramethylsilane as internal standard.

2.12. Statistical analysis

In vivo experiments and the enzymatic assays with varying DTT concentrations were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test when appropriate. The data of experiments with a fixed DTT concentration were analyzed by three-way analysis of variance. All other results were analyzed by two-way ANOVA, followed by Duncan's Multiple Range Test when appropriate. Differences between groups were considered to be significant when $P \leq 0.05$.

Table 1
IC_{50} (μM) values for δ-ALA-D inhibition by $PhSeCH_3$ and $C_4H_9C{\equiv}CSeCH_3$ and their reaction products with H_2O_2[a]

Compound	Rat (S_1)	Hog (P_{55})	Plant (S_1)
$PhSeCH_3$	>400	>400	>400
$PhSeCH_3 + H_2O_2$	104	61	97
$C_4H_9C{\equiv}CSeCH_3$	>400	>400	>400
$C_4H_9C{\equiv}CSeCH_3 + H_2O_2$	72	123	210

[a] Reaction of selenides with H_2O_2 was carried out for 1 h and the remaining H_2O_2 was eliminated by adding catalase (200 U). Enzyme pre-incubation with selenides or products from the reaction of selenides with H_2O_2 was started by adding the supernatant from rat (S_1) or partially purified hog liver δ-ALA-D (P_{55}) to a medium containing 84 mM potassium phosphate buffer (pH 6.4). For plant δ-ALA-D, supernatants from cucumber leaves (S_1) was added to a medium containing 50 mM Tris–HCl buffer (pH 9.0). The reaction was started 10 min later by adding ALA (2.5 mM). Data are expressed as the mean of five to seven independent experiments. S.E.M. was less than 15% of respective means.

3. Results

3.1. Selenocompounds × H_2O_2 × δ-ALA-D activity

The compounds phenyl methyl selenide ($PhSeCH_3$) and 1-hexynyl methyl selenide ($C_4H_9C{\equiv}CSeCH_3$) did not inhibit hepatic δ-ALA-D. However, the products of reaction of phenyl methyl selenide or 1-hexynyl selenide with H_2O_2 inhibited δ-ALA-D from rat liver with IC_{50} values in the micromolar range (Table 1). Statistical analysis indicated a significant ($P \leq 0.01$) interaction between selenide and H_2O_2, indicating that the reactions of the selenides with H_2O_2

generated more potent products that inhibited δ-ALA-D from rat liver.

δ-ALA-D was partially purified to assess if endogenous liver substances existing in S_1 would contribute to the inactivation of δ-ALA-D by these selenocompounds. The inhibitory effects of phenyl methyl selenide and 1-hexynyl methyl selenide on δ-ALA-D activity from hog (P_{55}) were similar to those obtained using S_1 (Table 1). As observed with S_1, the selenides did not inhibit the partially purified enzyme and the products of the reactions of these compounds with H_2O_2 inhibited ALA-D with IC_{50} values in the micromolar range (Table 1).

δ-ALA-D from mammals possesses at least two sites that contain cysteinyl residues. One of these sites binds zinc with relatively low affinity (B site), while the other binds zinc with relatively high affinity (A site) (Dent et al. 1990). In plants, the site homologous to mammalian B site is characterized by the substitution of cysteinyl residues by acidic amino acids, which apparently renders the enzyme less susceptible to oxidation (Jaffe et al., 1995). Similarly to that obtained with mammalian enzyme, the products of the reaction of the selenides with H_2O_2 inhibited δ-ALA-D from cucumber leaves. The IC_{50} values for δ-ALA-D inhibition by the products of reaction between H_2O_2 and phenyl methyl selenide and 1-hexynyl methyl selenide were in the micromolar range (Table 1).

The interaction between selenocompounds and –SH groups has been frequently reported (Tsen and Tappel, 1958; Ganther, 1968; Barbosa et al., 1998; Maciel et al., 2000). Moreover, selenoxides are rapidly reduced by glutathione (GSH), yielding oxidized glutathione and the corresponding selenide (Chen and Ziegler, 1994; Goeger and Ganther, 1994). Involvement of cysteinyl groups in δ-ALA-D inhibition by the products of the reaction between the selenides and H_2O_2 were examined by testing the effect of DTT on δ-ALA-D. Addition of DTT (2 mM) increased δ-ALA-D activity by 28–35% and protected δ-ALA-D from the inhibition caused by the products of reaction between the selenides and H_2O_2 (Fig. 1A,B). The increase in δ-ALA-D activity caused by DTT is due to reactivation of enzyme molecules that oxidized during tissue preparation. Similar protection by DTT against the inhibitory effects of products of reaction of selenides with H_2O_2 was observed when plant δ-ALA-D was used (data not shown).

3.2. ^{1}H-NMR studies for chemical characterization of the selenoxides

^{1}H-NMR studies were carried out in order to characterize the chemical structure of products of

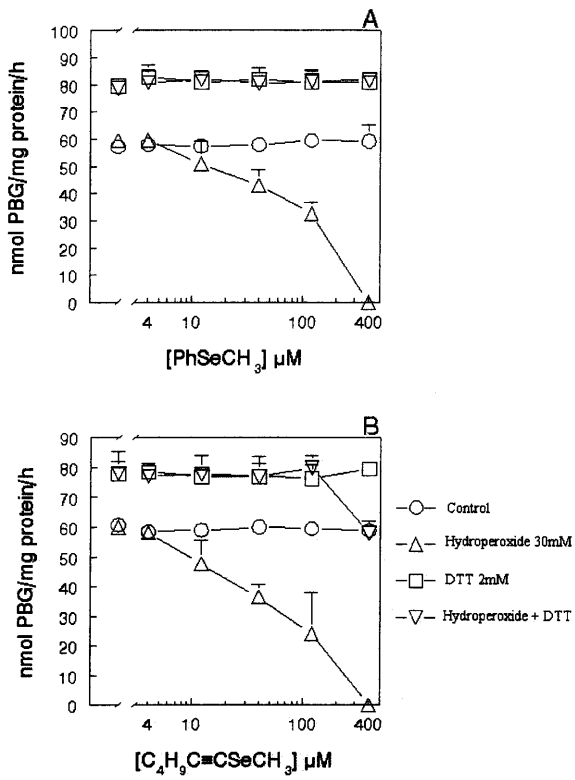


Fig. 1. Effect of $PhSeCH_3$ (A) and $C_4H_9C{\equiv}CSeCH_3$ (B), and of their products of reaction with H_2O_2 on δ-ALA-D from liver. Reaction of selenides with H_2O_2 was carried out for 1 h and the remaining H_2O_2 was eliminated by adding catalase (200 U). Enzyme pre-incubation with selenides or products from the reaction of selenides with H_2O_2 was started by adding the liver supernatant (S1) to a medium containing 84 mM potassium phosphate buffer (pH 6.4) in the absence or in the presence of 2 mM DTT. The δ-ALA-D reaction was started 10 min later by adding ALA (to give a final concentration of 2.5 mM and a volume of 0.25 ml). Data are expressed as mean ± S.E.M. for three independent experiments.

the reaction between phenyl methyl selenide and H_2O_2. Analysis revealed the presence of a single compound that corresponds to methyl phenyl selenoxide ($\delta = 2.63$ ppm (3H, s, $-CH_3$)) (Chen and Ziegler, 1994). The ^{1}H-NMR spectrum of the 1-hexynyl methyl selenide yielded a sign of methylene hydrogens linked to the selenium atom at $\delta = 2.26$ ppm (3H, s, $SeCH_3$) (Braga et al. 1993a,b). After reaction between 1-hexynyl methyl selenide and hydroperoxide, the ^{1}H-NMR spectrum revealed a sign of methylene hydrogens linked to the selenoxide group at $\delta = 2.55$ ppm (3H, s, $-CH_3$), indicating the formation of 1-hexynyl methyl selenoxide.

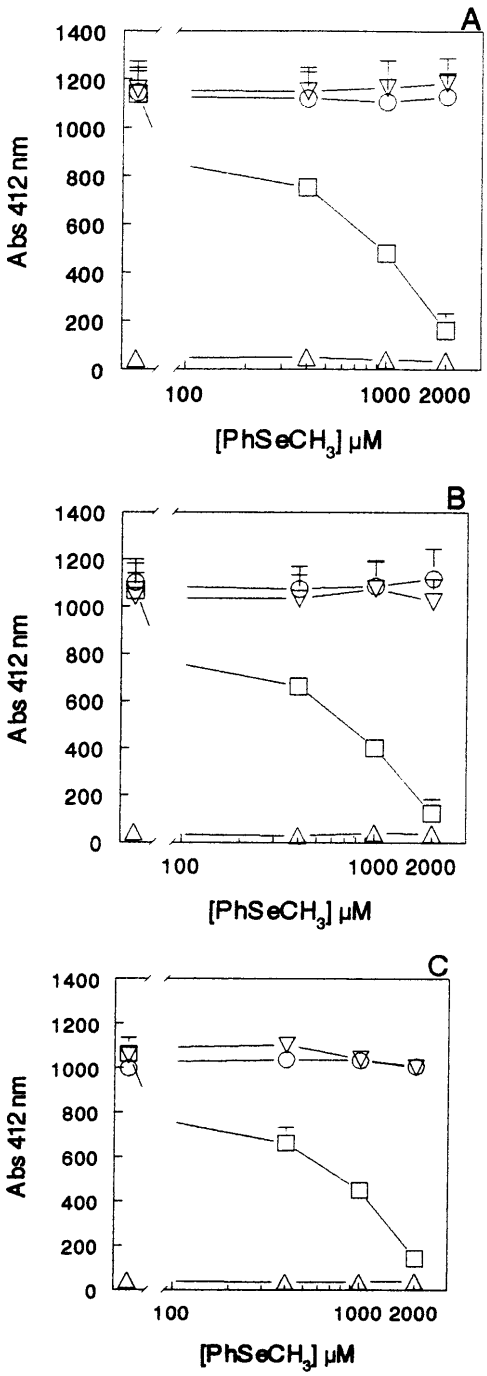


Fig. 2. (*Continued*)

3.3. Selenocompounds × H_2O_2 × DTT oxidation

Although the selenides did not affect DTT oxidation (Figs. 2 and 3), the products of reaction between phenyl methyl selenide and 1-hexynyl methyl selenide with H_2O_2 decreased the total amount of free –SH groups from DTT. Phenyl methyl selenoxide oxidized DTT immediately (at zero time) and stoichometrically, since 2 mM DTT was totally oxidized by 2 mM of this selenoxide (Fig. 2). The products of reaction between 1-hexynyl methyl selenide (2 mM) and H_2O_2 also oxidized practically all DTT. However, this was seen only after 30 min reaction (Fig. 3). Thus, the reactivity of these selenoxides toward –SH groups of DTT were quite different.

3.4. In vivo exposure to phenyl methyl selenide

Taking into account the fact that phenyl methyl selenide is a substrate for FMO (Chen and Ziegler, 1994) and the fact that its selenoxide inhibited δ-ALA-D activity, mice were exposed to

Fig. 2. Effect of $PhSeCH_3$ on the rate of DTT oxidation at 0 min (A), 30 min (B), and 60 min (C). Reaction of selenides with H_2O_2 was carried out for 1 h and the remaining H_2O_2 was decomposed by reaction with catalase (200 U) for 45 min (H_2O_2 + catalase). Parallel tubes with no H_2O_2 added (control), with no catalase added (H_2O_2), or with the addition of catalase (catalase) were also run. The reaction was started by adding DTT to a final concentration of 2 mM in a medium containing 84 mM potassium phosphate buffer (pH 6.4) and the selenide (0–2000 µM). Data are expressed as mean ± S.E.M. for three independent experiments: ○, control; △, H_2O_2; ▽, catalase (200 U); □, H_2O_2 + catalase.

phenyl methyl selenide in order to verify a possible bioactivation of this xenobiotic. Exposure to phenyl methyl selenide caused a significant decrease in weight gain and an increase in liver as percentage of body weight when compared with the control groups (Table 2). ALA-D activity decreased in the animals exposed to phenyl methyl selenide. Moreover, the addition of DTT (2 mM) to the enzymatic assay did not restore ALA-D activity in these animals. A parallel group was also exposed to 1-hexynyl selenide (500 μmol/kg/day) and the animals died within 3 days of treatment.

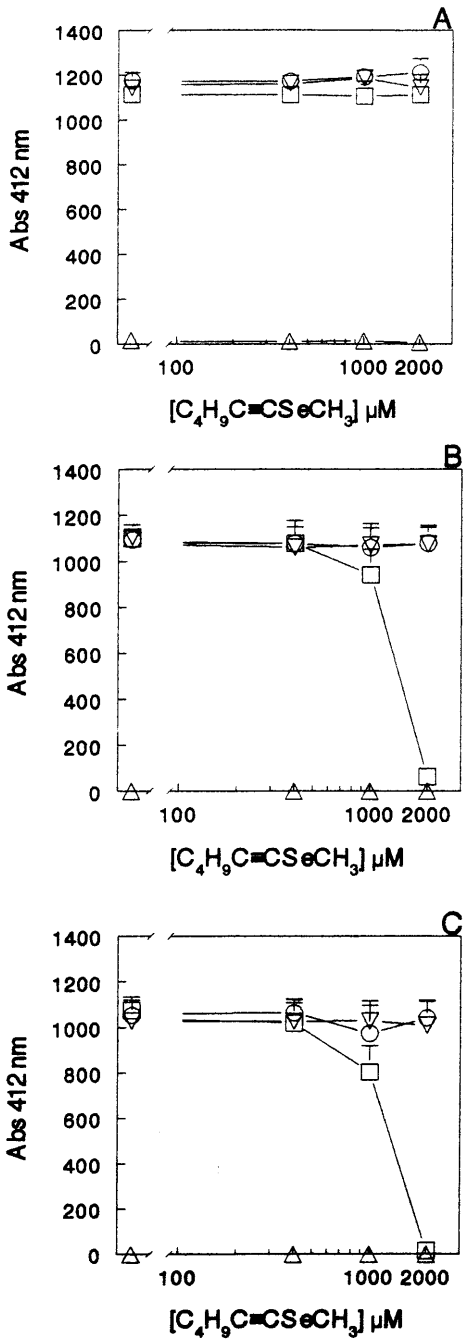


Fig. 3. (*Continued*)

4. Discussion

The present results demonstrate that the compounds phenyl methyl selenide and 1-hexynyl methyl selenide did not inhibit δ-ALA-D from a mammalian source, while the products of reaction of these selenides with H_2O_2 inhibited the enzyme activity with IC_{50} values in the micromolar range. Previous studies indicated that the reaction of some selenides with H_2O_2 produces their corresponding oxides (Chen and Ziegler, 1994). In accordance with this, the ^{1}H-NMR revealed the presence of the corresponding selenoxides after reaction between the selenides and H_2O_2. Consequently, the inhibition of δ-ALA-D can be ascribed to these oxidized forms of selenides.

The inhibition of δ-ALA-D by selenium compounds involves the oxidation of essential –SH groups of the enzyme, since DTT can counteract this inhibition. Other evidence supporting this view is the fact that the products of reaction between the selenides and H_2O_2 oxidized DTT. Previously, Chen and Ziegler (1994) demonstrated the reduction of selenoxides by low molecular

Fig. 3. Effect of $C_4H_9C{\equiv}CSeCH_3$ on the rate of DTT oxidation at 0 min (A), 30 min (B) and 60 min (C). Reaction of selenides with H_2O_2 was carried out for 1 h and the remaining H_2O_2 was decomposed by reaction with catalase (200 U) for 45 min (H_2O_2 + catalase). Parallel tubes with no H_2O_2 added (control), with no catalase added (H_2O_2), or with the addition of catalase (catalase) were also run. The reaction was started by adding DTT to a final concentration of 2 mM in a medium containing 84 mM potassium phosphate buffer (pH 6.4) and the selenide (0–2000 μM). Data are expressed as mean ± S.E.M. for three independent experiments: ○, control; △, H_2O_2; ▽, catalase (200 U); □, H_2O_2 + catalase.

weight thiols such as GSH, thioglycolate and thiocholine; however, to our knowledge, there are no data in literature reporting the reduction of selenoxides by sulfhydryl-containing proteins.

In plant δ-ALA-D, no cysteinyl residues are present in the region homologous to that involved in Zn^{2+} binding in mammalian δ-ALA-D and, in part, this may explain the lower sensitivity of plant enzyme to some oxidizing agents (Jaffe et al., 1995). However, plant enzyme is inactivated by iodoacetamide and *N*-ethylmaleimide, two classical sulfhydryl reagents, implying that cysteinyl residue(s) located at site(s) other than that equivalent to the Zn^{2+}-binding sites of the mammalian enzyme are important for δ-ALA-D activity in plants (Senior et al., 1996). In contrast to mammalian δ-ALA-D, which is inhibited by organic diselenides, the plant enzyme was not inhibited by diselenides, probably due to the fact that the inhibition caused by these compounds involves the oxidation of cysteinyl residues located at the B Zn^{2+}-binding site (Barbosa et al., 1998). The results of the present study suggest that the selenoxides inhibit plant enzyme, which is in accordance with the potent thiol oxidant properties of these compounds (Chen and Ziegler, 1994). Therefore, differing from diphenyl diselenide, selenoxides like iodoacetamide and *N*-ethylmaleimide oxidize the cysteinyl residue(s) of the plant enzyme located at site(s) other than that equivalent to the Zn^{2+}-binding sites of mammalian enzyme.

In contrast to that observed in vitro, DTT did not cause re-activation of inhibited δ-ALA-D activity in animals exposed to selenium. This may indicate that these compounds are reducing specifically the synthesis of this enzyme (the concentration of total protein was not changed by phenyl methyl selenide; data not shown). Alternatively, the oxidation caused by this organochalcogen on δ-ALA-D may modify the enzyme conformation in a such way that DTT did not reactivate the enzyme. In fact, δ-ALA-D has an octameric structure (Jaffe et al., 1995), and in the mammalian enzyme an intramonomer disulfide bond is apparently formed between cysteine 119 and 123 (Markham et al. 1993). However, it is possible that enzyme oxidation in vivo produces formation of a disulfide bond between δ-ALA-D monomers that may be not reduced by DTT.

Taking into account the role of FMO in oxidizing dialkyl- and alkylaryl-selenides (Ziegler et al., 1991), (Chen and Ziegler, 1994; Goeger and Ganther, 1994) it is plausible that their selenoxides are generated in vivo during the intoxication of mammals with these compounds. This may contribute to their toxicity through oxidation of sulfhydryl-containing enzymes like δ-ALA-D. Although the concentrations of H_2O_2 used to oxidize the selenides to their respective selenoxides were high and do not approach the hydroperoxide concentrations of biological fluids and tissues, the in vivo oxidation of these selenides is possible because a sophisticated enzymatic phenomenon exists in mammalian tissues. In the FMO catalytic mechanism, molecular oxygen oxidizes flavin to yield

Table 2
Effect of in vivo exposure to phenyl methyl selenide on δ-ALA-D activity in mice liver[a]

	45 days of treatment		30 days of treatment	
	Control	$PhSeCH_3$	Control	$PhSeCH_3$
Body weight (g)	41.8 ± 2.2	33.5 ± 2.5*	28.5 ± 2.9	20.1 ± 0.5*
Liver as a percentage of body weight	5.7 ± 0.6	12.6 ± 0.7*	5.9 ± 0.5	10.7 ± 0.5*
δ-ALA-D activity (nmol/PBG/h)	269.8 ± 10.2	95.4 ± 6.9*	241.3 ± 14.5	115.0 ± 8.2*
δ-ALA-D activity (DTT, 2mM)	329.0 ± 17.5	114.2 ± 8.5*	283.3 ± 17.2	128.5 ± 9.8*

[a] Mice were weighed daily and injected with DMSO (control) or $PhSeCH_3$ (500 μmol/kg) for 45 or 30 days and killed 24 h after the last injection. Tissue preparation and δ-ALA-D assay were as described in Section 2. Data are expressed as means ± S.E.M. ($n = 7$ for each group). * Significantly different from control.

peroxyflavin, which can oxidize the selenium atom (Ziegler, 1988).

Phenyl methyl selenide has chemopreventive activity against cancer in rodents (Ip et al., 1998), raising the possibility of therapeutic use of this compound by humans. Furthermore, the main metabolic product of inorganic selenium, dimethyl selenide, is also substrate for FMO (Goeger and Ganther, 1994) and its oxidation generates dimethyl selenoxide. Thus, exposure to high levels of dietary inorganic selenium can result in the formation of dimethyl selenoxide, which may have toxic effects to sulfhydryl-containing enzymes, like δ-ALA-D. Accordingly, there are some points of evidence indicating that exposure to selenium causes perturbation of heme synthesis in vivo both in animals and humans (Holnes et al., 1989; Khan et al., 1993; Maciel et al. 2000; Jaques-Silva et al. 2001), and can be associated with elevated urine porphyrins in humans (Ransome, 1961).

In summary, these results suggest that selenoxides can oxidize sulfhydryl-containing proteins and that mammalian δ-ALA-D is a potential molecular target for selenides as a consequence of their bioactivation to selenoxides by FMO.

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