Bioactivation of Chemopreventive Selenocysteine *Se*-Conjugates and Related Amino Acids by Amino Acid Oxidases Novel Route of Metabolism of Selenoamino Acids

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Several selenocysteine *Se*-conjugates have been shown to possess potent chemopreventive activity in animal models for chemical carcinogenesis. As a mechanism of action, β-elimination reactions to form chemopreventive selenols, ammonia, and pyruvate has been proposed. The enzymes involved in these β-elimination reactions, however, have been partially elucidated. Next to cysteine conjugate β-lyases, as yet unidentified non-pyridoxal-5′-phosphate-dependent enzymes also appear to be involved in cytosolic β-elimination reactions. In the present study, it was investigated whether amino acid oxidases contribute to the bioactivation of selenocysteine *Se*-conjugates. Using purified L-amino acid oxidase from *Crotalus adamanteus* as a model enzyme, significant β-elimination activities were indeed observed upon incubation with *Se*-methylselenocysteine (K_m, 195 μM; k_{cat}, 48 min^{-1}), *Se*-allylselenocysteine (K_m, 608 μM; k_{cat}, 34 min^{-1}), *Se*-phenylselenocysteine (K_m, 107 μM; k_{cat}, 57 min^{-1}) and *Se*-benzylselenocysteine (K_m, 59 μM; k_{cat}, 13 min^{-1}). For all selenocysteine *Se*-conjugates tested, the rate of pyruvate formation was comparable to that of hydrogen peroxide, one of the products of oxidative deamination. The fact that addition of catalase did not alter pyruvate formation indicated that the β-elimination reaction observed was not mediated by selenoxidation/syn-elimination due to the hydrogen peroxide formed via the oxidative deamination pathway. Using D-amino acid oxidase from porcine kidney and D-SeCys conjugates similar results were obtained. To delineate whether mammalian L-amino acid oxidases are also able to catalyze β-elimination of selenocysteine *Se*-conjugates, rat renal cytosol was fractionated and screened for β-elimination and oxidative deamination activities. One of the fractions isolated displayed oxidative deamination activity with several amino acids and cysteine *S*-conjugates. With selenocysteine *Se*-conjugates as substrates, however, this fraction displayed both oxidative deamination and β-elimination activities, when incubated in the presence of aminoxyacetic acid to block contribution of pyridoxal-5′-phosphate-dependent enzymes. The potential significance of this novel bioactivation route for the chemopreventive activity of selenocysteine *Se*-conjugates is discussed.

Introduction

Over the past decade, numerous studies have reported that low molecular weight selenium compounds exert significant chemopreventive activities in animal tumor models (*1*, *2*). Generation of monomethylated selenium metabolites is believed to mediate the chemopreventive activities of selenomethionine, selenobetaine, sodium selenite, methyl selenocyanate, and *Se*-methyl-L-selenocysteine. The observation that longer alkyl chain homologues showed a higher chemopreventive potency than methyl selenocyanate indicated that antitumor activity is not restricted to monomethylated selenium species, in principle allowing the design of more powerful anticancer selenium compounds (*1*). Very recently, *Se*-allyl-D,L-selenocysteine appeared to be a more potent chemopreventive agent when compared to *Se*-methyl-D,L-selenocysteine (*3*). Dietary administration of 2 ppm *Se*-allyl-D,L-selenocysteine resulted in an almost 90% reduction of methylnitrosourea-induced mammary tumors in rats, making it one of the most potent chemopreventive agents identified. *Se*-(*n*-propyl)-D,L-selenocysteine showed the same potency as *Se*-methyl-D,L-selenocysteine.

As a mechanism of action of selenocysteine *Se*-conjugates (SeCys[1] conjugates), bioactivation by β-elimination reactions has been proposed, resulting in the formation of pyruvate, ammonia, and chemopreventive selenols (*1*, *3*, *4*). The enzymes involved in these bioactivation reactions, however, are only partially character-

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[1] Abbreviations: AAO, amino acid oxidase; AOAA, aminooxyacetic acid; APCI, atmospheric pressure chemical ionization; CFTE-Cys, *S*-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine; FPLC, fast-protein liquid chromatography; HPA, *p*-hydroxyphenylacetic acid; HRP, horseradish peroxidase; KMB, α-keto-γ-methiolbutyrate; PLP, pyridoxal-5′-phosphate; SeCys, selenocysteine *Se*.

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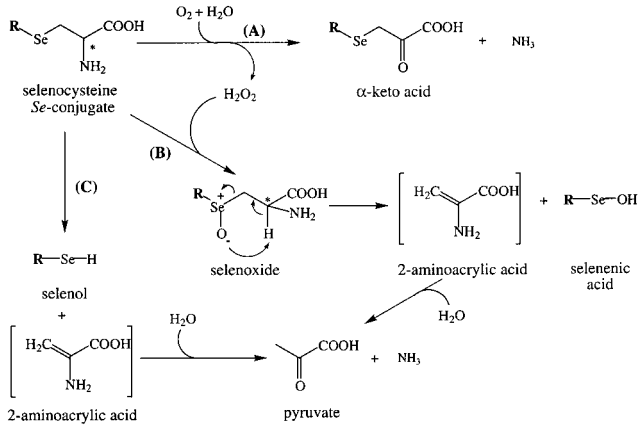

Figure 1. Hypothetical mechanisms of oxidative deamination and β-elimination of SeCys conjugates by amino acid oxidase. Reaction A: oxidative deamination leading to α-keto acids, ammonia and hydrogen peroxide. Reaction B: selenoxidation by hydrogen peroxide and subsequent syn-elimination of selenoxide leading to selenenic acids and 2-aminoacrylic acid, as described previously for flavin-containing monooxygenases (*8*). 2-aminoacrylic acid rapidly hydrolyses into pyruvate and ammonia. Reaction C: direct β-elimination leading to a selenol and 2-aminoacrylic acid, the latter of which hydrolyses into pyruvate and ammonia.

ized. Identification and localization of these enzymes may help to elucidate in which tissues SeCys conjugates may exert chemopreventive activities. Previously, high β-elimination activities of SeCys conjugates were shown in both rat and human renal cytosol (*4*, *5*). Strong inhibition by aminooxyacetic acid (AOAA) indicated the involvement of pyridoxal-5′-phosphate (PLP)-dependent enzymes, such as cysteine conjugate β-lyases (*6*). The high activities observed upon incubation of SeCys conjugates with highly purified rat renal cysteine conjugate β-lyase/glutamine transaminase K revealed that this protein contributes significantly to the renal activity (*7*). However, the fact that AOAA only partially inhibited β-elimination of some SeCys conjugates, such as *Se*-benzyl-L-selenocysteine, indicated that non-PLP-dependent enzymes also appeared to be involved in the β-elimination of SeCys conjugates in rat renal cytosol (*6*). As an alternative bioactivation pathway, cytosolic selenoxidation followed by syn-elimination was proposed (*6*). Very recently, this hypothesis was confirmed by the observation that selenoxidation of SeCys conjugates by flavin-containing monooxygenases in rat liver microsomes and recombinantly expressed human flavin-containing monooxygenases indeed resulted in spontaneous syn-elimination, leading to the formation of pyruvate, ammonia and selenenic acids (*8*).

In the present study, it was studied whether amino acid oxidases (AAOs) may also be involved in cytosolic β-elimination reactions of SeCys conjugates. AAOs typically catalyze oxidative deamination reactions of amino acids resulting in the formation of corresponding α-keto acids and hydrogen peroxide (Figure 1, route A). Several racemic SeCys conjugates, such as *Se*-carboxymethyl-D,L-selenocysteine, *Se*-(1-carboxyethyl)-, *Se*-(1-carboxypropyl)-, and *Se*-(2-carboxyethyl)-D,L-selenocysteine have been reported to be substrates for L-AAO from *Crotalus adamanteus* and D-aspartate oxidase from beef kidney (*9*−*11*). In the present study, we investigated whether the hydrogen peroxide generated during oxidative deamination of SeCys conjugates is able to selenoxidize SeCys conjugates, which subsequently would be followed by a syn-elimination reaction as described above (Figure 1, route B).

Next to oxidative deamination, however, it has been demonstrated that for a limited number of substrates AAOs may also catalyze β-eliminations (*12*). Using β-chloro-D-alanine and purified hog kidney D-AAO it was delineated that under anaerobic conditions only pyruvate and chloride were formed. Under a 100% oxygen atmosphere, almost exclusively chloropyruvate was formed, whereas mixtures of pyruvate and chloropyruvate were observed at intermediate oxygen levels. The total amount of product (pyruvate and chloropyruvate) formed was independent of the oxygen tension, which is indicative for a common intermediate of the substrate and the enzyme. Similar results were obtained when β-chloro-L-alanine was incubated with L-AAO from *Crotalus adamanteus* (*12*). β-Elimination reactions by hog kidney D-AAO were also observed with α-amino-β-chlorobutyrate, β-bromo-D,L-alanine and β-cyano-D-alanine as substrates (*13*−*15*). Therefore, in analogy with these compounds a similar mechanism may be applicable in case of SeCys conjugates as substrates (Figure 1, route C). This pathway would result in formation of pyruvate, ammonia and selenol, instead of the selenenic acid, which is formed if β-elimination is mediated by the selenoxidation pathway (Figure 1, route A).

In the present study, the bioactivation of chemopreventive SeCys conjugates and structurally related cysteine *S*-conjugates via oxidative deamination and β-elimination reactions was investigated, using purified L-AAO from *Crotalus adamanteus* as a model enzyme. Furthermore, it was delineated whether pyruvate formed resulted from selenoxidation by hydrogen peroxide and/or from direct β-elimination. Finally, the possible involvement of AAO(s) in pyruvate formation from SeCys conjugates by rat renal cytosol was investigated by fractionation of rat renal cytosol by anion-exchange chromatography.

Experimental Procedures

Reagents. Aminooxyacetic acid (AOAA; carboxymethoxylamine hemihydrochloride), diphenyldiselenide, and pyruvate were from Aldrich (Beerse, Belgium). *S*-Benzyl-L-cysteine, L-phenylalanine, D-phenylalanine, and *o*-phenylenediamine were purchased from Janssen Chimica (Geel, Belgium). Catalase from beef liver (25 000 units/mg) was obtained from Boehringer (Mannheim, Germany). Hydrogen peroxide was from J. T. Baker (Deventer, The Netherlands). D-AAO from porcine kidney (0.14 units/mg), L-AAO type I from *Crotalus adamanteus* (0.46 units/mg), *p*-hydroxyphenylacetic acid (HPA), *Se*-methyl-L-selenocysteine, β-chloro-L-alanine, α-keto-γ-methiolbutyrate (KMB), and horseradish peroxidase (HRP) type I from horseradish (116 unints/mg) were purchased from Sigma (St. Louis, MO). *S*-(2-Chloro-1,1,2-trifluoroethyl)-L-cysteine (CFTE-Cys) was synthesized as described previously (*16*). SeCys conjugates were prepared as described previously (*4*, *5*). Methylphenylselenide was prepared by reduction of diphenyldiselenide with $NaBH_4$ followed by methylation with diazomethane (*4*). All other chemicals were of the highest grade commercially available.

Biotransformation of SeCys Conjugates and Cysteine *S*-Conjugates by L-AAO. First, incubations were performed to investigate the time-dependence of formation of pyruvate and hydrogen peroxide at 0.5 mM SeCys conjugate. Second, enzyme kinetic parameters were determined by a fixed incubation time and with varying substrate concentrations. All incubations described below were performed in triplicate and were also

performed in absence of L-AAO to correct for nonenzymatic reactions.

Pyruvate Formation. Incubations (200 μL) containing 0.5 mM SeCys conjugate and 1 μg of L-AAO from *Crotalus adamanteus* in 100 mM potassium phosphate buffer (pH 7.2) were incubated at 37 °C (to dissolve the substrates 5 M sodium hydroxide was added until pH 8−9, after dissolution 5 M HCl was added until pH 7.2). After 0, 5, 10, 15, or 20 min, 20 μL of 0.3 M zinc sulfate was added to terminate the reaction and the samples were placed on ice. In case of enzyme kinetic analysis, substrates were incubated for 20 min at 10 different concentrations ranging from 0 to 500 μM. Enzyme kinetic parameters were obtained using Hanes-Woolf plots. After termination of the reactions by zinc sulfate, samples were centrifuged for 15 min at 4000*g* and the amount of pyruvate in the supernatant was measured after derivatization with *o*-phenylenediamine, as described previously (*17*). Incubations were also performed in the presence of 1% catalase (sufficient to scavenge at least 500 μM of hydrogen peroxide) to delineate the role of hydrogen peroxide-mediated selenoxidation in the pyruvate formation. To exclude contribution of contaminating PLP-dependent cysteine conjugate β-lyases, incubations were also performed in the presence of 1 mM AOAA.

Hydrogen Peroxide Formation. To quantify formation of hydrogen peroxide, incubations were also performed in the presence of 0.94 mM HPA and 28.5 μg/mL HRP, leading to the in situ oxidation of HPA to the fluorescent product 2,2′-dihydroxybiphenyl-5,5′-diacetate (*18*). The presence of HRP and HPA did not alter the pyruvate formation. In this case, a 80 μL incubation sample was added to a quartz cuvette and diluted with 720 μL of 100 mM potassium phosphate buffer (pH 7.2) and the fluorescence was detected at an excitation wavelength of 323 nm and an emission wavelength of 400 nm. The amount of hydrogen peroxide was analyzed using standard curves.

Identification of Products of L-AAO and *Se*-Phenyl-L-selenocysteine and Assessment of Stoichiometry. The stoichiometry of products was determined by performing a large-scale incubation of L-AAO and *Se*-phenyl-L-selenocysteine as model substrate. Incubations (3 mL) containing 0.94 mM HPA, 85.7 μg of HRP, 0.5 mM *Se*-phenyl-L-selenocysteine and 15 μg of L-AAO in 100 mM potassium phosphate buffer (pH 7.2) were incubated at 37 °C (to dissolve the substrates 5 M sodium hydroxide was added until pH 8−9, after dissolution 5 M HCl was added until pH 7.2). At several time points 450 μL aliquots were mixed with 50 μL of 0.3 M $ZnSO_4$, placed on ice and centrifuged at 4000*g* for 15 min. Incubations were performed in triplicate, and incubations without L-AAO were performed to correct for nonenzymatic degradation. The amount of pyruvate and hydrogen peroxide was measured as described above. Ammonia was measured using an ammonium kit of Sigma (St. Louis, MO) based on the reductive amination of 2-oxoglutarate by glutamate dehydrogenase and NADPH. Consumption of *Se*-phenyl-L-selenocysteine was measured by a modified method of Wang et al. (*19*), using GC-ECD, as follows: To a solution containing 60 μL of sample, 10 μL of 4 mM L-phenylalanine (as an internal standard), 30 μL of 2,2,2-trifluoroethanol, and 10 μL of pyridine was added 10 μL of ethylchloroformate. After 2 min, 500 μL of heptane was added and the derivatized products were extracted and analyzed by GC-ECD as described below.

Selenium-containing products were analyzed by GC-MS and LC-APCI-MS/MS, as described below. For GC-MS analysis, samples were acidified by 2 N hydrochloric acid, extracted by ethyl acetate, and methylated by ethereal diazomethane. For LC-APCI-MS/MS-analysis, samples were deproteinized by zinc sulfate after which the supernatant is applied directly to LC-APCI-MS/MS, as described below (see Analytical Equipment). Selenium-containing products were also determined in incubations in absence of HPA and HRP, and in incubations in the presence of 1% catalase.

Incubations with D-AAO. Specific activities of oxidative deamination and β-elimination of *Se*-(*n*-propyl)-D-selenocysteine and *Se*-(4-methylphenyl)-D-selenocysteine were determined as described for L-AAO (see above). However, in these experiments a final concentration of 1 mg/mL D-AAO from porcine kidney was used.

To investigate the role of selenoxidation, incubations were performed with D-AAO and D-phenylalanine in the presence of *Se*-phenyl-L-selenocysteine. Incubations (1 mL) containing 0.94 mM HPA, 28.5 μg of HRP, 0.25 mM *Se*-phenyl-L-selenocysteine, 1 mM D-phenylalanine, and 2.5 mg D-AAO were incubated at 37 °C (to dissolve the substrates 5 M sodium hydroxide was added until pH 8−9, after dissolution 5 M HCl was added until pH 7.2). At several time points 200 μL aliquots were mixed with 20 μL of 0.3 M $ZnSO_4$, placed on ice and centrifuged for 15 min at 4000*g*. Pyruvate and hydrogen peroxide were measured as described above. All incubations were performed in triplicate, and incubations without D-AAO were performed to correct for nonenzymatic degradation.

Fractionation of Rat Kidney Cytosol by Anion-Exchange Fast-Protein Liquid Chromatography (FPLC). Rat kidney cytosol (nondialyzed) was prepared as previously described (*17*) and was fractionated by FPLC using a Mono Q anion-exchange column (Pharmacia Biotech, Uppsala, Sweden), as described by Commandeur et al. (*7*). Thirty fractions of 2 mL were collected and analyzed directly for enzyme activity. All incubations described below were performed in triplicate and were also performed in absence of fraction to correct for nonenzymatic reactions.

Activity profiles of β-elimination and oxidative deamination were determined by incubating fractions with *Se*-phenyl-L-selenocysteine. In case of β-elimination assessment, 10 μL of FPLC-fraction was added to 50 μL of 50 mM sodium borate buffer (pH 8.6), containing 0.6 mM *Se*-phenyl-L-selenocysteine and 0.6 mM KMB. After 20 min, 250 mL of 0.14% *o*-phenylenediamine in 3 N HCl was added. Samples were heated for 45 min at 60 °C and analyzed as described above. To investigate the role of PLP-dependent cysteine conjugate β-lyases, incubations were also performed (a) in the presence of 1 mM AOAA and in absence of KMB and (b) at various KMB concentrations with *Se*-(4-methylbenzyl)-L-selenocysteine as a substrate (0.5 mM). In case of oxidative deamination assessment, incubations (200 μL) containing 0.94 mM HPA, 5.7 μg of HRP, 0.5 mM *Se*-phenyl-L-selenocysteine, and 20 μL of FPLC fraction in 50 mM sodium borate buffer (pH 8.6) were incubated at 37 °C. After 20 min, 20 μL 0.3 M $ZnSO_4$ was added to terminate the reaction and the samples were placed on ice. Samples were centrifuged for 15 min at 4000*g* and the amount of hydrogen peroxide was measured as described above. The active fraction number 12, containing L-AAO (L-α-hydroxy acid oxidase), was analyzed for β-elimination activity in the presence of AOAA and in absence of KMB, and oxidative deamination activity both at pH 7.2 (100 mM potassium phosphate buffer) and 8.6 (as described above). Substrates were L-phenylalanine, *Se*-phenyl-L-selenocysteine, *S*-phenyl-L-cysteine, *Se*-benzyl-L-selenocysteine, *S*-benzyl-L-cysteine, CFTE-Cys, and β-chloro-L-alanine (to dissolve the substrates in 100 mM potassium phosphate buffer, pH 7.2, 5 M sodium hydroxide was added until pH 8−9, after dissolution 5 M HCl was added until pH 7.2). Protein concentration of the fractions was determined with the Bio-Rad protein kit (Hercules, CA).

Analytical Equipment. *GC-MS.* GC-MS analyses were performed on a HP 5890 gas chromatograph equipped with a 25 m BPX5 column (0.25 mm i.d., 0.25 μm film thickness, SGE, Amstelveen, The Netherlands) coupled to a Hewlett-Packard MSD 5970 mass spectrometer (ion source, electron impact ionization, electron energy of 70 eV). Temperatures of the injection port and transfer line were 270 °C. The column temperature was programmed from 60 °C (2 min) to 270 °C (20 °C/min) and kept at 270 °C for 10 min. Full scanning analyses were performed in the range of *m*/*z* 30−650. Using these GC-MS conditions, the following retention times and mass spectra were recorded:

Methylphenylselenide. Phenylselenol was detected as the methylated derivative due to diazomethane treatment. Reten-

tion time, 5.4 min; mass spectrum *m/z* (relative intensity, selenium isotope, assignment) 172 (85, ^{80}Se, $M^{\bullet+}$), 157 (100, ^{80}Se, $M^{\bullet+} - CH_3$), 117 (20), 91 (45), 77 (47, $C_6H_5^+$), 51 (56).

Methylated Phenylselenoacetic Acid. Phenylselenoacetic acid was detected as the methylated derivative due to diazomethane treatment. Retention time, 8.2 min; mass spectrum *m/z* (relative intensity, selenium isotope, assignment) 230 (98,^{80}Se, $M^{\bullet+}$), 171 (59, ^{80}Se, $M^{\bullet+} - COOCH_3$), 157 (61, ^{80}Se, $C_6H_5Se^+$), 91 (100), 77 (52, $C_6H_5^+$).

Dimethylated 2-Oxo-3-phenylselenopropionic Acid (Phenylselenopyruvate). Due to keto−enol tautomerization of 2-oxo-3-phenylselenopropionic acid, methylation by diazomethane appeared to be not only at the carboxyl position, but also at the α-keto−enol position, as observed previously for 3-mercapto-2-oxopropionic acid *S*-conjugates and α-ketoacids of cysteine *S*-conjugates (*20*). Retention time, 10.1 min (and 10.2 min); mass spectrum *m/z* (relative intensity, selenium isotope, assignment) 272 (94, ^{80}Se, $M^{\bullet+}$), 157 (100, ^{80}Se, $C_6H_5Se^+$), 115 (40, $M^{\bullet+} - C_6H_5Se$), 91 (60), 77 (63, $C_6H_5^+$), 59 (44, $COOCH_3^+$).

Diphenyldiselenide. Retention time, 11.3 min; mass spectrum *m/z* (relative intensity, selenium isotope, assignment) 314 (67, ^{80}Se, $M^{\bullet+}$), 234 (16, ^{80}Se, $M^{\bullet+}$- Se), 157 (100, ^{80}Se, $C_6H_5Se^+$), 117 (19), 77 (66, $C_6H_5^+$), 51 (39).

GC-ECD. Samples were injected onto an automated Hewlettt Packard 5890 series II gas chromatograph equipped with a 15 mCi ^{63}Ni electron-capture detector. GC separation was achieved on a 25 m HP-5 column (0.32 mm i.d., 0.25 μm film thickness, Hewlettt Packard, Amstelveen, The Netherlands). The temperature of the GC oven was programmed from 160 °C (1 min) to 270 °C, at 10 °C/min, and kept at 270 °C for 10 min. The temperatures of the injector and detector were 270 and 300 °C, respectively. The helium column flow rate was 3.2 mL/min. Under these conditions the retention times were: L-phenylalanine derivative 6.46 min and *Se*-phenyl-L-selenocysteine derivative 9.57 min.

LC-APCI-MS/MS. The LC system consisted of an Inertsil ODS-2 column (5 μm particle, 150 × 4.60 mm, Phenomenex, Amstelveen, The Netherlands) which was eluted at a flow rate of 1 mL/min using the following gradient: initially 2% B for 3 min followed by an increase to 92% B over 30 min; at 35 min returned to 2% B in 1 min. Mobile phase A consisted of 1% acetonitrile in water, and mobile phase B was 100% acetonitrile. The eluent from the HPLC column was analyzed using an UV-detector, set at 220 and 254 nm, and subsequently passed into the ionization region of the mass spectrometer without flow splitting. Mass spectrometric detection was carried out with a Finnigan LCQ-DECA mass spectrometer (ThermoQuest, San Jose, CA) operating in the negative ion mode using an APCI interface probe. A potential of 5 kV was applied to the APCI corona discharge. High purity (99.999%, Praxair) N_2 was used as drying gas, with a flow of 300 L/h). Full scanning analyses were performed in the range of *m/z* 150−650. MS/MS spectra were recorded of ions at *m/z* 243 for 2-oxo-3-phenylselenopropionic acid (phenylselenopyruvate) and at *m/z* 215 for phenylselenoacetic acid.

Results

Biotransformation of SeCys Conjugates by L-AAO. Incubating SeCys conjugates and L-AAO from *Crotalus adamanteus* produced both hydrogen peroxide and pyruvate, both being linear up to 30 min (Figure 2, panels A and B). On the basis of these results, subsequent enzyme kinetic studies were performed using incubation times of 20 min

The enzyme kinetic parameters of L-AAO from *Crotalus adamanteus* for hydrogen peroxide and pyruvate formation from eight substrates are shown in Table 1. From these results, it appeared that all substrates tested were metabolized by oxidative deamination (Figure 1, route A), as measured by hydrogen peroxide formation.

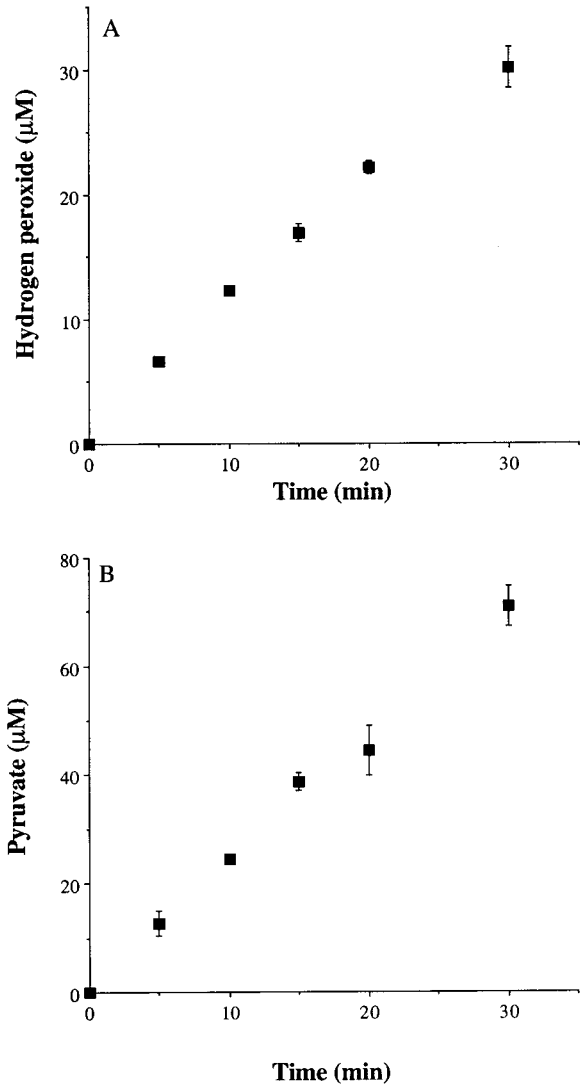

Figure 2. Time-dependent formation of hydrogen peroxide (A) and pyruvate (B) upon incubation of *Se*-phenyl-L-selenocysteine (0.5 mM) with L-AAO from *Crotalus adamanteus* (5 μg/mL). Experiments were performed as described in the Experimental Procedures. Results are presented as mean value ± SD for three preparations.

Activities exceeded that of the known substrate L-phenylalanine. The highest catalytic efficiency (k_{cat}/K_m) for hydrogen peroxide formation was obtained for *Se*-benzyl-L-selenocysteine (0.394 ± 0.099 min^{-1} μM^{-1}), *Se*-methyl-L-selenocysteine (0.318 ± 0.016 min^{-1} μM^{-1}), *Se*-phenyl-L-selenocysteine (0.307 ± 0.043 min^{-1} μM^{-1}), and CTFE-Cys (0.276 ± 0.058 min^{-1} μM^{-1}). *Se*-Allyl-L-selenocysteine was the SeCys conjugate with the lowest k_{cat}/K_m-value. *S*-Benzyl-L-cysteine and *S*-phenyl-L-cysteine showed 5-fold higher K_m-values, when compared to their selenium analogues (Table 1).

In contrast to oxidative deamination which was observed with all substrates, formation of pyruvate was restricted exclusively to the SeCys conjugates tested (Table 1). None of the cysteine *S*-conjugates were metabolized by β-elimination by L-AAO from *Crotalus adamanteus*. For all SeCys conjugates except *Se*-phenyl-L-selenocysteine, catalytic efficiencies (k_{cat}/K_m) for pyruvate formation were slightly lower than those for hydrogen peroxide formation. For the SeCys conjugates the catalytic efficiency (k_{cat}/K_m) for pyruvate formation decreased

Table 1. Enzyme Kinetic Parameters of L-AAO from *Crotalus adamanteus* for Oxidative Deamination (hydrogen peroxide formation) and β-Elimination (pyruvate formation) toward Various Amino Acids[a]

substrate	reaction	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m ($min^{-1}\,\mu M^{-1}$)
Se-benzyl-L-selenocysteine	oxidative deamination	7.83 ± 0.54	20 ± 4	0.394 ± 0.099
	β-elimination	13.10 ± 0.49	59 ± 7	0.221 ± 0.049
S-benzyl-L-cysteine	oxidative deamination	7.09 ± 0.27	126 ± 13	0.056 ± 0.004
	β-elimination	ND	ND	ND
Se-phenyl-L-selenocysteine	oxidative deamination	12.50 ± 0.32	41 ± 6	0.307 ± 0.043
	β-elimination	57.02 ± 4.06	107 ± 21	0.532 ± 0.084
S-phenyl-L-cysteine	oxidative deamination	6.08 ± 0.47	204 ± 35	0.030 ± 0.007
	β-elimination	ND	ND	ND
Se-methyl-L-selenocysteine	oxidative deamination	32.48 ± 0.77	102 ± 6	0.318 ± 0.016
	β-elimination	47.96 ± 2.12	195 ± 18	0.247 ± 0.012
Se-allyl-L-selenocysteine	oxidative deamination	17.16 ± 0.56	190 ± 13	0.090 ± 0.003
	β-elimination	34.17 ± 3.57	608 ± 95	0.056 ± 0.002
CTFE-Cys	oxidative deamination	10.93 ± 0.46	40 ± 5	0.276 ± 0.058
	β-elimination	ND	ND	ND
L-phenylalanine	oxidative deamination	12.85 ± 0.34	125 ± 10	0.103 ± 0.005
	β-elimination	ND	ND	ND

[a] Experiments were performed as described in the Experimental Procedures. k_{cat}, K_m, and k_{cat}/K_m values were obtained from Hanes-Woolf plots. Results are presented as mean values ± SD for three preparations. ND not detectable.

Table 2. Stoichiometry of Metabolism of *Se*-Phenyl-L-selenocysteine by L-AAO from *Crotalus adamanteus*[a]

reaction	specific activity (nmol/min/mg L-AAO)
substrate consumption	626 ± 168
ammonia formation	596 ± 46
pyruvate formation	466 ± 14
hydrogen peroxide formation	200 ± 6

[a] Experiments were performed as described in the Experimental Procedures. Results are presented as mean value ± SD for three preparations.

in the order: *Se*-phenyl-L-selenocysteine > *Se*-benzyl-L-selenocysteine ≈ *Se*-methyl-L-selenocysteine > *Se*-allyl-L-selenocysteine.

Stoichiometry of Products Formed from *Se*-Phenyl-L-selenocysteine. For *Se*-phenyl-L-selenocysteine, selected as a model substrate, the stoichiometry of the products formed upon incubation with L-AAO was quantified (Table 2). As expected, the specific activity of substrate consumption (626 ± 168 nmol/min/mg) equaled the specific activity of formation of ammonia (596 ± 46 nmol/min/mg) which is formed both via the β-elimination and oxidative deamination pathways, Figure 1. The specific activity of pyruvate formation, 466 ± 14 nmol/min/mg, indicated that for this SeCys conjugate, approximately 70−75% proceeds via the β-elimination pathway. In line with this, the specific activity of hydrogen peroxide formation, reflecting the oxidative deamination pathway, indeed equals to approximately 30% of the total *Se*-phenyl-L-selenocysteine consumption.

As shown in Table 3, the addition of 1 mM AOAA did not significantly alter the formation of pyruvate, thus excluding contamination of commercial L-AAO by PLP-dependent cysteine conjugate β-lyases. Neither was hydrogen peroxide formation influenced by AOAA. When the incubations were performed in the presence of 1% catalase, hydrogen peroxide formation could not be measured anymore indicating complete decomposition of hydrogen peroxide. However, pyruvate formation was not affected by the presence of catalase, indicating that β-elimination of *Se*-phenyl-L-selenocysteine is not mediated via selenoxidation/syn-elimination by hydrogen peroxide formed during oxidative deamination (Figure 1, routes A and B). This conclusion was further supported

Table 3. Effect of AOAA and Catalase on Hydrogen Peroxide and Pyruvate Formation from *Se*-Phenyl-L-selenocysteine in the Presence of L-AAO from *Crotalus adamanteus*, and the Effect of Hydrogen Peroxide Generated from D-AAO/D-Phenylalanine upon Pyruvate Formation from *Se*-Phenyl-L-selenocysteine[a]

incubation mixture	hydrogen peroxide (nmol/min/mg)	pyruvate (nmol/min/mg)
L-AAO	182 ± 8	474 ± 4
+ AOAA	198 ± 10	466 ± 20
+ catalase	ND	490 ± 8
D-AAO/D-phenylalanine	194 ± 10	ND
+ *Se*-phenyl-L-selenocysteine		ND

[a] Experiments were performed as described in the Experimental Procedures. Results are presented as mean values ± SD for three preparations.

by incubations of *Se*-phenyl-L-selenocysteine in the presence of a hydrogen peroxide generating system consisting of D-phenylalanine and D-amino acid oxidase (Table 3). While hydrogen peroxide formation equaled that observed in incubations of *Se*-phenyl-L-selenocysteine in the presence of L-AAO, not a trace of pyruvate formation was noticed.

No products were observed when *Se*-phenyl-L-selenocysteine or L-phenylalanine was incubated in the presence of D-AAO, indicating absolute stereospecificity of this enzyme, as described previously (*21*).

Identification of Selenium-Containing Products by Mass Spectrometry. GC-MS analysis of methylated extracts of incubations of *Se*-phenyl-L-selenocysteine with L-AAO showed five selenium-containing products as indicated by the characteristic selenium-isotope patterns. The first selenium-containing product, at a retention time of 5.4 min (peak I, Figure 3) showed a molecular ion at *m*/*z* 172 (^{80}Se isotope) and a fragment at *m*/*z* 157, apparently due to loss of a methyl-radical (Figure 4A). Comparison with a synthetical reference revealed that this product was methylphenylselenide, formed by methylation of phenylselenol by diazomethane. A second selenium-containing product had a retention time of 8.2 min (peak II, Figure 3) and a molecular ion at *m*/*z* 230 (^{80}Se isotope) (Figure 4B). This mass is consistent with the molecular weight of methylated phenylselenoacetic acid, the product resulting from oxidative decarboxylation

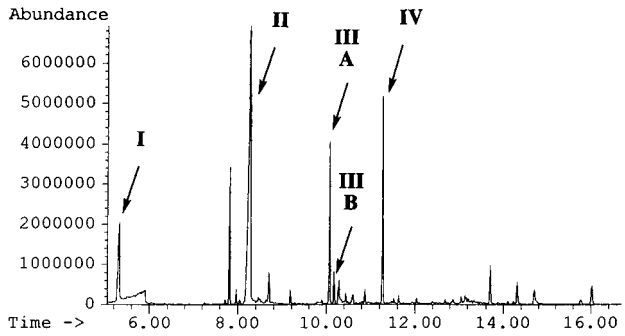

Figure 3. Total ion current (TIC) chromatogram of an incubation of *Se*-phenyl-L-selenocysteine with L-AAO. Samples were acidified, extracted and derivatized with ethereal diazomethane as described in "Experimental Procedures". Five selenium-containing metabolites were detected as indicated by their characteristic selenium-isotope patterns. Peak I represents methylphenylselenide, II methylated phenylselenoacetic acid, III dimethylated 2-oxo-3-phenylselenopropionic acid (cis- and trans-isomers) and IV diphenyldiselenide. Corresponding mass spectra are depicted in Figure 4.

of 2-oxo-3-phenylselenopropionic acid by hydrogen peroxide. In incubations in the presence of catalase and HRP/HPA, this product was not detectable, indicative for the involvement of hydrogen peroxide. The identity of phenylselenoacetic acid as a product was confirmed by LC-APCI-MS analyses (negative ion mode) of an underivatized sample showing a selenium-containing product with a retention time of 3.3 min with an ion at *m/z* 215 $[M - H]^-$ (highest mass and ^{80}Se isotope) consistent to the molecular weight of phenylselenoacetic acid. MS/MS analysis of the ion at *m/z* 215 showed a product ion at *m/z* 171 that is likely produced by decarboxylation (data not shown). Two other selenium-containing peaks (peak IIIA and IIIB, Figure 3) occurring at retention times 10.1 and 10.2 min had comparable mass spectra with a molecular ion at *m/z* 272 (^{80}Se isotope) (Figure 4C). These mass spectra most likely correspond to cis- and trans-isomers of dimethylated 2-oxo-3-phenylselenopropionic acid (phenylselenopyruvate), which is the product resulting from oxidative deamination. Due to keto−enol tautomerization of 2-oxo-3-phenylselenopropionic acid, methylation by diazomethane occurs both at the carboxyl position and at the enol-oxygen position, as previously observed in case of 3-mercapto-2-oxo-propionic acid *S*-conjugates, the α-ketoacids formed from cysteine *S*-conjugates (*20*). The identity of 2-oxo-3-phenylselenopropionic acid was also confirmed by LC-APCI-MS analyses (negative ion mode) of an underivatized reaction mixture. A selenium-containing product eluted at 4.5 min with an ion at *m/z* 243 $[M - H]^-$ (highest mass and ^{80}Se isotope), which is corresponding to the molecular weight of 2-oxo-3-phenylselenopropionic acid. MS/MS analysis of the ion at *m/z* 243 showed a product ion at *m/z* 157 corresponding to the phenylseleno-moiety (data not shown). The fourth product identified by GC-MS analysis was diphenyl diselenide (Figure 3, peak IV, and Figure 4D), according to its retention time (11.3 min) and mass spectrum (*m/z* 314 and ^{80}Se isotope) identical with commercially available reference compound. Diphenyl diselenide is expected to result from autoxidation of phenylselenol formed during the β-elimination reaction.

GC-MS analysis of methylated extracts of incubations of *Se*-methyl-L-selenocysteine and *Se*-allyl-L-selenocysteine with L-AAO showed only the presence of the corresponding dimethylated α-keto acids. In these incubations, the corresponding selenols and diselenides could not be detected as products. In incubations performed in

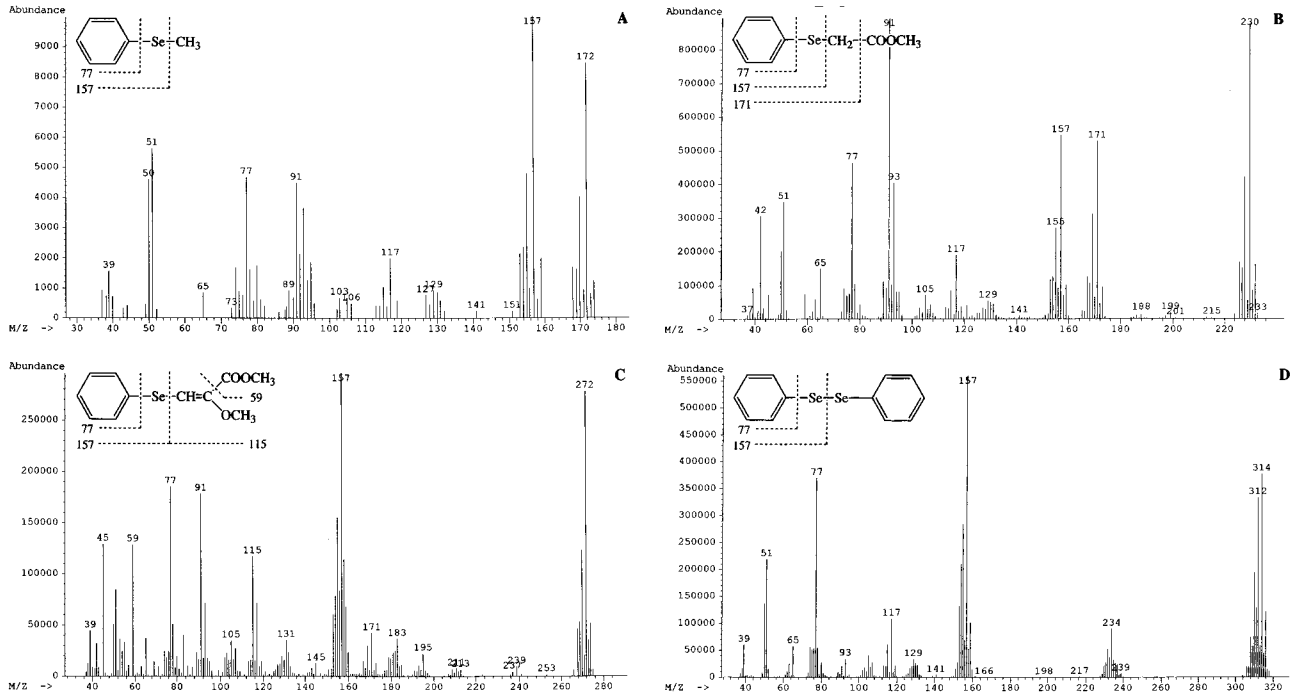

Figure 4. Mass spectra of an incubation of *Se*-phenyl-L-selenocysteine with L-AAO. Samples were acidified, extracted and derivatized with ethereal diazomethane as described in the Experimental Procedures. (A) Peak I represent methylphenylselenide (5.4 min, *m/z* 172), (B) peak II methylated phenylselenoacetic acid (8.2 min, *m/z* 230), (C) peak IIIA dimethylated 2-oxo-3-phenylselenopropionic acid (phenylselenopyruvate), and (D) peak IV diphenyldiselenide. Due to keto−enol tautomerization of 2-oxo-3-phenylselenopropionic acid, methylation by diazomethane occurred not only at the carboxyl position, but also at the α-keto−enol position, as observed previously for 3-mercapto-2-oxopropionic acid *S*-conjugates, α-ketoacids of cysteine *S*-conjugates (*20*). Fragmentation patterns and experimentals are described in the Experimental Procedures.

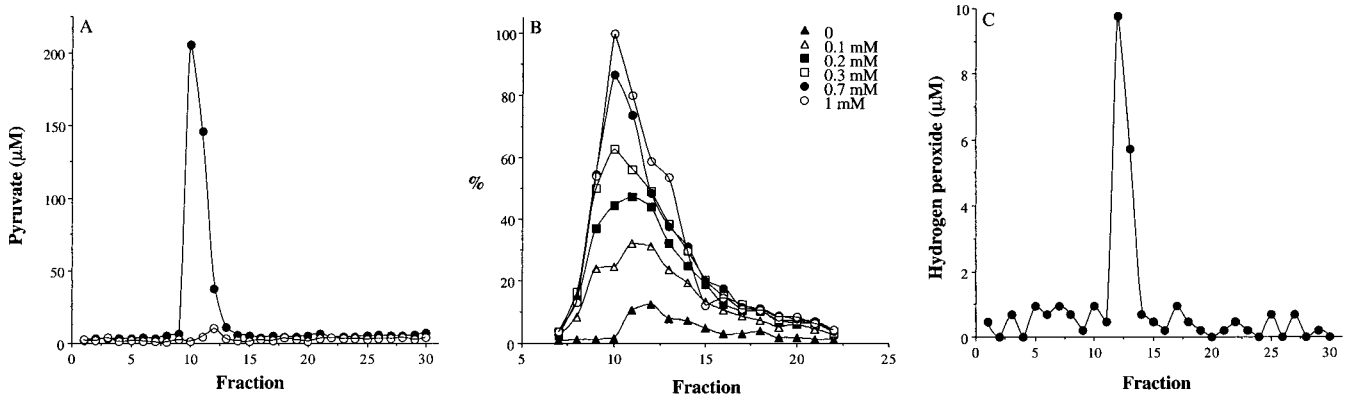

Figure 5. Activity profiles of β-elimination and oxidative deamination with SeCys conjugates in FPLC-fractions from rat renal cytosol applied to a Mono Q anion-exchange column. (A) β-Elimination of *Se*-phenyl-L-selenocysteine with FPLC-fractions (closed symbols), and in the presence of AOAA (open symbols). (B) β-Elimination of *Se*-(4-methylbenzyl)-L-selenocysteine with FPLC-fractions at various KMB concentrations. (C) Oxidative deamination of *Se*-phenyl-L-selenocysteine with FPLC-fractions. Experiments were performed as described in the Experimental Procedures.

Table 4. Specific Activity for Oxidative Deamination (hydrogen peroxide formation) and β-Elimination (pyruvate formation) toward Various Amino Acids in FPLC-Fraction 12[a]

	oxidative deamination		β-elimination	
Substrate	pH 8.6	pH 7.2	pH 8.6	pH 7.2
Se-benzyl-L-selenocysteine	4.11 ± 0.21	1.63 ± 0.17	4.97 ± 1.07	3.92 ± 0.04
S-benzyl-L-cysteine	0.69 ± 0.02	0.06 ± (2×10^{-3})	ND	ND
Se-phenyl-L-selenocysteine	1.62 ± 0.07	0.32 ± 0.03	1.13 ± 0.20	1.17 ± 0.21
S-phenyl-L-cysteine	0.49 ± 0.02	0.02 ± (8×10^{-5})	ND	ND
CTFE-Cys	0.47 ± 0.03	0.03 ± (1×10^{-3})	ND	ND
L-phenylalanine	0.59 ± 0.01	ND	ND	ND
β-chloro-L-alanine	0.32 ± 0.02	0.03 ± (1×10^{-3})	ND	ND

[a] Experiments were performed as described in the Experimental Procedures. Pyruvate determination was performed in the presence of 1 mM AOAA. Specific activities are presented in nanomoles per minute per milligram of protein. Results are presented as mean values ± SD for three preparations. ND, not detectable.

the presence of catalase, to prevent oxidation of methyl- and allylselenol, the corresponding selenols were also not detectable.

Fractionation of Rat Kidney Cytosol by Anion-Exchange FPLC. Rat kidney cytosol was fractionated using Mono Q anion-exchange FPLC, and 2 mL fractions were collected. The fractions were screened for β-elimination activity, measured as pyruvate formation, with *Se*-phenyl-L-selenocysteine as a substrate in the presence of 0.5 mM KMB, a known cofactor of PLP-dependent cysteine conjugate β-lyase enzymes (Figure 5A). The highest β-elimination activity was observed in fraction 10, corresponding to rat kidney glutamine transaminase K/cysteine conjugate β-lyase enzyme (*7*). In presence of AOAA, a well-known inhibitor of PLP-dependent enzymes, however, the β-elimination activity was highest in fraction 12 (Figure 5A). The β-elimination activity in fraction 10 was completely blocked by AOAA, indicating full dependence of PLP-dependent enzymes in this fraction. β-Elimination activity in fraction 12 was only partially inhibited by AOAA. The same pattern was observed using different concentrations of KMB, a cofactor of PLP-dependent cysteine conjugate β-lyase enzymes, and *Se*-(4-methylbenzyl)-L-selenocysteine as a substrate (Figure 5B). In presence of relatively high KMB concentrations (>0.2 mM), the highest β-elimination activity is present in fraction 10 and the activity profile is similar to Figure 5A. However, at lower KMB concentrations (0.1−0.2 mM), the highest β-elimination activity was present in fractions 11 and 12. In the absence of KMB, fraction 12 displayed the highest β-elimination activity and the activity profile was similar to the one obtained in the presence of AOAA (Figure 5A). Furthermore, the highest oxidative deamination, measured as hydrogen peroxide formation, was observed in fraction 12, while in fraction 10 no significant hydrogen peroxide was detected (Figure 5C). The oxidative deamination and β-elimination of several amino acids was determined in the presence of AOAA in fraction 12 at pH 7.2 and 8.6 (Table 4). These results show that all amino acids were oxidatively deaminated and that the specific activity is lower at pH 7.2 than at pH 8.6. Furthermore, the specific activity of oxidative deamination was much higher for SeCys conjugates than for the corresponding sulfur analogues. Formation of pyruvate from SeCys conjugates by β-elimination in the presence of AOAA was again only observed for the SeCys conjugates. The specific activity of β-elimination for *Se*-benzyl-L-selenocysteine was higher than for *Se*-phenyl-L-selenocysteine at both pH 7.2 and pH 8.6.

Biotransformation of SeCys Conjugates by D-AAO. Experiments with *Se*-(*n*-propyl)-D-selenocysteine and *Se*-(4-methylphenyl)-D-selenocysteine showed that D-amino acid oxidase from porcine kidney catalyzed both the oxidative deamination and β-elimination of these substrates (Table 5). In case of *Se*-(*n*-propyl)-D-selenocysteine, activity of oxidative deamination and β-elimination were comparable, while for *Se*-(4-methylphenyl)-D-selenocysteine β-elimination was the major pathway accounting for 75% of the total biotransformation by D-AAO. The corresponding L-enantiomers were metabolized by L-AAO from *Crotalus adamanteus* via oxidative deamination and β-elimination (Table 5). Both enzymes were highly stereoselective since the L-SeCys conjugates

Table 5. Specific Activity for Oxidative Deamination (hydrogen peroxide formation) and *β*-Elimination (pyruvate formation) of L-SeCys Conjugates by L-AAO from *Crotalus adamanteus* and D-SeCys Conjugates by D-AAO from Porcine Kidney[a]

substrate	hydrogen peroxide (nmol/min/mg)	pyruvate (nmol/min/mg)
Se-(*n*-propyl)-L-selenocysteine	453 ± 26	384 ± 74
Se-(*n*-propyl)-D-selenocysteine	2.0 ± 0.16	1.8 ± 0.08
Se-(4-methylphenyl)-L-selenocysteine	530 ± 16	551 ± 32
Se-(4-methylphenyl)-D-selenocysteine	2.0 ± 0.24	6.0 ± 0.51

[a] Experiments were performed as described in the Experimental Procedures. Results are presented as mean values ± SD for three preparations.

were exclusively metabolized by L-AAO and the D-SeCys conjugates were exclusively metabolized by D-AAO. Specific activities of L-AAO and D-AAO, however, should not be compared quantitatively because of the different source of the enzymes.

Discussion

The present study shows that both pyruvate and hydrogen peroxide is formed upon incubations of purified L-AAO from *Crotalus adamanteus* and purified D-AAO from porcine kidney with L-SeCys conjugates and D-SeCys conjugates, respectively. Pyruvate formation appears to proceed via a direct *β*-elimination implicating formation of selenols as products (Figure 1, route C), instead of being mediated by selenoxidation by hydrogen peroxide (Figure 1, route B). With *Se*-phenyl-L-selenocysteine, formation of phenylselenol was indeed confirmed by analysis of selenium-containing products by GC-MS (Figure 3). For the chemopreventive SeCys conjugates *Se*-methyl-L-selenocysteine and *Se*-allyl-L-selenocysteine, however, formation of the anticipated selenols could not be demonstrated yet due to their high volatility and/or their oxidation by hydrogen peroxide. Addition of catalase to prevent this oxidation was unsuccessful. It might therefore be possible that in the presence of catalase and hydrogen peroxide methyl- and allylselenols are rapidly oxidized similar as has been described for small alcohols, such as methanol and allyl alcohol (*35*).

In contrast to the SeCys conjugates, in case of the corresponding sulfur analogues no pyruvate formation was observed. A similar result was observed previously when SeCys conjugates and the corresponding *S*-conjugates were incubated with highly purified PLP-dependent rat renal cysteine conjugate *β*-lyase/glutamine transaminase K (*7*). Possible explanations for the higher susceptibility of SeCys conjugates for *β*-elimination may be the weaker bond strength of the C−Se bond (234 kJ/mol) versus the C−S bond (272 kJ/mol) (*22*) and/or a more facile *β*-proton abstraction of the selenocysteine moiety (*23*). Interestingly, CFTE-Cys, which is one of the best substrates known for PLP-dependent cysteine conjugate *β*-lyases, was also not *β*-eliminated at all by L-AAO, indicating that different factors determine substrate selectivity when compared to PLP-dependent enzymes. Although no pyruvate formation was detected, all cysteine *S*-conjugates tested were actively metabolized by oxidative deamination reactions, as reflected by hydrogen peroxide formation. Consistent with previous studies with different SeCys conjugates and cysteine *S*-conjugates (*11*, *24*), the cysteine *S*-conjugates showed up to 10-fold lower deamination activities when compared to corresponding SeCys conjugates (Table 1).

To investigate whether mammalian amino acid oxidase is also able to catalyze *β*-elimination reactions of SeCys conjugates, rat kidney cytosol was fractionated by anion-exchange chromatography. When SeCys conjugates were incubated in the presence of AOAA, to inhibit PLP-dependent enzymes, one of the fractions displayed both pyruvate formation and hydrogen peroxide formation (Figure 5). In presence of phenylalanine and cysteine *S*-conjugates, this fraction only displayed hydrogen peroxide formation. These results strongly suggest that amino acid oxidase is involved in the non-PLP-dependent *β*-elimination of SeCys conjugates in rat renal cytosol. Rat renal cytosolic L-AAO, also called α-hydroxy acid oxidase or B isoform, is a flavin-dependent peroxisomal oxidase which can metabolize both L-α-hydroxy acids and L-amino acids to α-keto acids (*25*). The oxidative deamination was higher at pH 8.6 than at pH 7.2 for all substrates (Table 4), which is consistent with the pH-optimum for rat renal L-AAO, known to range between pH 8.0 and 10 depending on the amino acid used (*26*). Similar to L-AAO from *Crotalus adamanteus*, specific activities of oxidative deamination in rat renal cytosol were higher for the SeCys conjugates than for the corresponding sulfur analogues. *β*-Elimination by cytosolic rat renal L-AAO was only obtained for the SeCys conjugates.

As yet little is known about the substrate specificity of cytosolic rat renal L-AAO. The enzyme is frequently called long-chain α-hydroxy acid oxidase, because good substrates for this enzyme are those containing benzyl- or alkyl-groups of 2−5 carbons (*25*). Consistent to this structure−activity relationship, *Se*-benzyl-L-selenocysteine appeared to be an excellent substrate for cytosolic rat renal L-AAO, showing the highest activities of both oxidative deamination and *β*-elimination (Table 4). Therefore, the relatively poor inhibition (21%) of *β*-elimination activity in rat renal cytosol by AOAA, as described previously (*6*), may be explained by the high activity of L-AAO for this substrate in combination with a relatively low activity of cysteine conjugate *β*-lyase/glutamine transaminase K (*7*). In case of *Se*-phenyl-L-selenocysteine, *β*-elimination in rat renal cytosol appeared to be mediated almost completely (95%) by PLP-dependent enzymes (*6*). This SeCys conjugate indeed showed a very high activity when incubated with PLP-dependent cysteine conjugate *β*-lyase/glutamine transaminase K (*7*), whereas in the present study a relatively low activity is observed with rat renal L-AAO (Table 4).

The present study indicates that in addition to PLP-dependent cysteine conjugate *β*-lyases and flavin-containing monooxygenases, AAOs may contribute to the bioactivation of SeCys conjugates, as summarized in Figure 6. Both *β*-elimination and oxidative deamination pathways may be relevant for chemopreventive activity,

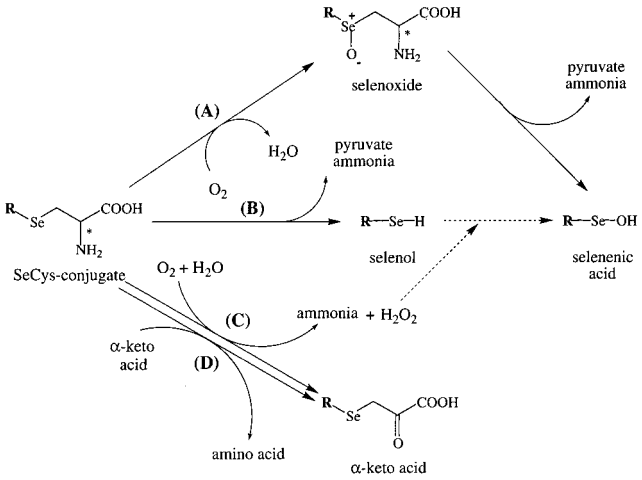

Figure 6. Identified enzymes active in the bioactivation of SeCys conjugates. Reaction A: selenoxidation by flavin-containing monooxygenases followed by a spontaneous syn-elimination of the selenoxide. Reaction B: β-elimination by PLP-dependent cysteine conjugate β-lyases and amino acid oxidases. Reaction C: oxidative deamination by amino acid oxidases. Reaction D: transamination by PLP-dependent cysteine conjugate β-lyases.

because both selenols and hydrogen peroxide are known to possess antitumor activity (*1*, *27*, *28*). Furthermore, hydrogen peroxide may oxidize the selenols formed to selenenic acids and seleninic acids. Seleninic acids were recently shown to be chemopreventive both in vivo as in vitro (*29*). As mode of action of selenium compounds, interference with the Zn-homeostasis has been proposed recently (*2*, *30*−*32*). One of the enzymes strongly regulated by the Zn-homeostasis is caspase-3, which plays an important role in apoptosis (*33*). Release of inhibitory zinc by complexing to selenols was proposed as a mechanism leading to apoptosis (*2*). Recently, it was shown that zinc-release from metallothionein by benzeneselenol was dramatically enhanced by addition of *tert*-butylhydroperoxide (*31*). It is therefore tempting to speculate that parallel formation of selenols and hydrogen peroxide by amino acid oxidase, as observed in the present study, may be involved in chemopreventive activity of SeCys conjugates, however, this remains to be established.

Recently, three SeCys conjugates were shown to be very active in the chemoprevention against methylnitrosourea-induced mammary tumors in rats (*3*). The SeCys conjugates tested, however, were racemic mixtures of L- and D-SeCys conjugates. In the present study it was shown that D-AAO is also able to catalyze the β-elimination as well as the oxidative deamination of D-SeCys conjugates. Therefore, it may be anticipated that D-AAOs may also be involved in case racemic mixtures of SeCys conjugates are administered. In this respect, it is interesting to note that recently a novel cancer gene therapy paradigm was exploited based on the expression of D-AAO in tumor cells in which the peroxisomal targeting sequence was removed (*34*). Exposure of these cells to D-alanine caused cytotoxicity mediated by oxidative stress at concentrations that were nontoxic to parental cells. Using D-isomers of SeCys conjugates in this therapy would not only lead to oxidative stress due to hydrogen peroxide, but also to the generation of pharmacologically active selenols.

In conclusion, the present study indicates that, next to flavin-containing monooxygenase enzymes and cysteine conjugate β-lyases, such as glutamine transaminase K, AAOs may be involved in the bioactivation of SeCys conjugates. The relative contribution of these enzymes in the chemopreventive activity of SeCys conjugates remains to be established, however. In case of the involvement of reactive metabolites of SeCys conjugates in the chemopreventive activity, tissue distribution of these enzymes may determine the organ selectivity of SeCys conjugates as chemopreventive agents.

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