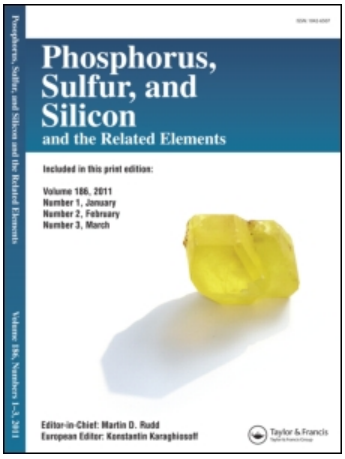

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BIOCHEMICAL ASPECTS OF SELENIUM AMINO ACIDS AND SELENIUM PEPTIDES

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Abstract We discovered peculiar enzymes participating in selenium amino acid metabolism: selenocysteine β-lyase occurring in mammalian tissues and bacteria, and D-selenocystine α,β-lyase occurring in *Clostridium sticklandii*. We here describe enzymological properties and reaction mechanisms of these enzymes. We synthesized the selenocysteine analogues of *Neurospora crassa* copper metallothionein as well as four diastereoisomers of glutathione. Their physicochemical properties and reactivity with peroxides are also described.

INTRODUCTION

Seleium is an essential micronutrient for mammals, birds, fish and several bacteria.[1] The physiological functions of selenium in mammals and birds can be attributed mostly to the action of glutathione peroxidase that contains essential selenocysteine residue in the polypeptide chain. Several microbial enzymes also have been demonstrated to contain selenocysteine residue in their polypeptide chains: the selenocsyteine residue plays an integral role in the catalysis.[1] Various other natural selenium amino acids occur in a free state. Selenium amino acids are thought to be synthesized through the analogous pathway to the sulfur counterparts. Various enzymes acting on sulfur amino acids (e.g. mammalian cystathionine γ-lyase and bacterial methionine γ-lyase) work on the selenium analogues, although enzymes that act specifically on selenium compounds have been considered. We here describe enzymological aspects of selenium amino acids metabolism. Selenocysteine-containing peptides are expected to show interesting physiological functions, but their facile synthesis is not available. We describe also synthesis of four selenium analogues of glutathione (i.e. glutaselenone), and *Neurospora crassa* copper metallothionein (i.e. metalloselenonein), and their properties.

SYNTHESIS OF SELENOCYSTEINE

Selenomethionine has been demonstrated in wheat and some other grains, but there are few reports of the occurrence of selenocysteine and selenocystine.[2] In mammalian tissues, selenocysteine synthesis was assumed to be analogous to that of cysteine. Selenomethionine serves as a better substrate than methionine for ATP:L-methionine *S*-adenosyltransferase of rabbit liver and other sources. *Se*-Adenosylselenomethionine was shown to be an efficient methyl donor in various methylation systems. We have shown that cystathionine β-synthase of rat liver catalyzes the selenocystathionine formation (equation 1) in addition to the cystathionine synthesis (equation 2).[3]

L-serine + L-selenohomocysteine → L-selenocystathionine + H_2O [1]

L-serine + L-homocysteine → L-cystathionine + H_2O [2]

Selenohomocysteine is as susceptible as homocysteine: the relative V_{max} value is about 0.7. L-Homocysteine inhibits the selenocystathionine synthesis in the presence of 5 mM L-serine as a competitive inhibitor for L-selenohomocysteine, and L-selenohomocysteine also inhibits the cystathionine synthesis in the same manner. The K_i values are substantially consistent with the K_m values, indicating that both reactions 1 and 2 are carried out at the same active site. Cystathionine γ-lyase can catalyze α,β- elimination of L-cystine in addition to α,γ-elimination of L-cystathionine. We have found that the α,β-elimination of L-cystathionine (equation 3) proceeds much more slowly (<3%) than the

L-cystathionine + H_2O → pyruvate + L-homocysteine + NH_3 [3]

L-cystathionine + H_2O → α-ketobutyrate + L-cysteine + NH_3 [4]

L-selenocystathionine + H_2O → pyruvate + L-selenohomocysteine + NH_3 [5]

L-selenocystathionine + H_2O → α-ketobutyrate + L-selenocysteine + NH_3 [6]

α,γ-elimination (equation 4). However, the α,β-elimination of selenocystathionine (equation 5) proceeds at a comparable rate with the α,γ-elimination of the same substrate (equation 6). Cystathionine γ-lyase can eliminate further both the amino acids formed from selenocystathionine and cystathionine by elimination reactions, though slowly. All the selenium amino acids are decomposed 2.5-3 times more rapidly than the corresponding sulfur analogues. Cystathionine β-synthase of chicken liver catalyzes synthesis of cysteine from serine and H_2S (equation 7). The rat liver enzyme also catalyzes the reaction at a rate of 12% of cystathionine synthesis (equation 2), but selenocysteine cannot be synthesized directly from L-serine and H_2Se (equation 8).

L-serine + H_2S → L-cysteine + H_2O [7]

L-serine + H_2Se → L-selenocysteine + H_2O [8]

This is probably due to low reactivity of selenide as a substituent donor in replacement reaction as demonstrated with *O*-acetylserine (thiol)-lyase.

OCCURRENCE OF SELENOCYSTEINE β-LYASE AND ITS ENZYMOLOGICAL PROPERTIES

Selenocysteine is synthesized by the coupled reactions with cystathionine β-synthase (EC 4.2.1.22) and cystathionine γ-lyase (EC 4.4.1.1) purified from rat liver, and also by the reaction system with a rat liver homogenate. However, the selenocysteine synthesis proceeds far less efficiently with the homogenate than with the purified enzymes. We have found that this is due to the presence of a novel enzyme in the homogenate that decomposes specifically selenocysteine into alanine and H_2Se.[4] This reaction apparently is a reduction, but the enzyme inherently catalyzes the removal of elemental selenium from L-selenocysteine; the formation of H_2Se is due to a spontaneous reduction of a product, elemental selenium, with the substrate, selenocysteine unreacted. Thus, the enzyme has been termed selenocysteine β-lyase, or systematically selenocysteine selenium-lyase (alanine-forming).

Selenocysteine β-lyase is distributed widely in mammalian tissues. The enzyme activities of livers and kidneys are higher than those of other tissues in several animals. Significant activity is found in pancreas and adrenal, but no activity occurs in blood and fat. The enzyme has been demonstrated also in various bacterial strains such as *Citrobacter freundii*, *Alcaligenes viscolactis*, and *Pseudomonas alkanolytica*.[5] However, no significant activity was found in yeasts and fungi.

Selenocysteine β-lyase has been purified to homogeneity from pig liver[4] and *Citrobacter freundii*[6] and characterized (TABLE I). The bacterial enzyme is different markedly from the mammalian enzyme in its physicochemical properties and amino acid composition. In contrast, both the enzymes are very similar in their enzymological properties: both contain pyridoxal 5'-phosphate (pyridoxal-P) as a coenzyme, exhibit strict specificity for L-selenocysteine, and similar K_m values for the substrates. L-Cysteine behaves as a competitive inhibitor against L-selenocysteine for both the enzymes. Based on the K_m value for L-selenocysteine and the K_i value for L-cysteine, the enzyme probably acts on selenocysteine very slowly *in vivo*, because the concentration of selenocysteine in the tissues is lower than the K_m value. However, the

total activity of enzyme is most likely sufficient to metabolize a small amount of selenocysteine in the tissues. The localization and compartmentation of the enzyme, the

TABLE I Properties of selenocysteine β-lyase.

Properties	Pig liver enzyme	Citrobacter freundii enzyme
Molecular weight	93,000	63,000
Subunit structure	Dimer	Monomer
Isoelectric point	5.9	6.6
Absorption maxima	280, 420	280, 420
Pyridoxal-P content (mol per mol of enzyme)	1	1
Optimum pH	8.4	7.0
Substrate (K_m)	L-Selenocysteine (0.8 mM)	L-selenocysteine (0.8 mM)
Competitive inhibitor (K_i)	L-Cysteine (1.0 mM)	L-Cysteine (0.65 mM)
Inactivation by abortive transamination	Yes	Yes
Irreversible inactivation by β-chloroalanine	Yes	Yes

substrate, and the inhibitors probably affect the enzyme activity *in vivo*.

Selenomethionine and selenocysteine are toxic for animals, and H_2Se is the most toxic selenium compounds so far studied. Selenocysteine can be synthesized from selenomethionine derived from a diet. H_2Se is produced from selenomethionine through selenocysteine by catalysis of selenocysteine β-lyase. The lack of specificity of the enzymes acting on the biosynthetic pathway of cysteine from methionine (e.g. cystathionine γ-lyase and cystathionine β-synthase) and the presence of selenocysteine β-lyase may contribute in part to the selenium toxicity.

REACTION MECHANISM OF SELENOCYSTEINE β-LYASE

The selenocysteine β-lyase reaction is exceptional among those of the pyridoxal-P enzymes so far studied. The enzyme resembles bacterial aspartate β-decarboxylase (EC 4.1.1.12) and kynureninase (EC 3.7.1.3) in the reaction mechanism where a moiety binding to C_3 of the substrate is cleaved to produce alanine. We have proposed mechanism of the reactions catalyzed by selenocysteine β-lyase.[7] The selenohydryl group of selenocysteine is substantially in an anionic form under the physiological conditions because its pK_a is 5.2. Cysteine is not a substrate of selenocysteine β-lyase, but inhibits the enzyme reaction competitively with selenocysteine between pH 7.0 and 9.0. In this pH range, a thiol of cysteine is dissociated at least partially because the pKa of the thiol of cysteine is about 8.8. Thus, the difference of selenol and thiol in their enzymatic reactivities is not derived from that in their dissociation states. The deuterium isotope effect at the α position indicates that an α-hydrogen release occurs in the enzyme reaction and is rate limiting. The α-hydrogen of selenocysteine is abstracted by a base at the enzyme active site, and then selenium is removed in an elemental form. L-Cysteine can bind the enzyme active site, but elemental sulfur is not removed from a cysteine-pyridoxal-P aldimine complex due to a strong bond dissociation energy between the β carbon and sulfur of cysteine, which is stronger than that between the β carbon and selenium of selenocysteine (for C-S, 272 kJ/mol; for C-Se, 243 kJ/mol), as found in various organic reactions such as the reactions of episulfides and episelenides.

We have studied the selenocysteine β-lyase reactions in deuterium oxide to show deuterium incorporation into alanine. The ^1H and ^{13}C-NMR spectra of alanine produced indicate the formation of [β-$^1H_1$] and [β-$^1H_2$]alanines. Therefore, in addition to the incorporation of one deuterium atom into the β position of alanine after removal of elemental selenium, one of the two β-hydrogen atoms of selenocysteine is exchanged with a solvent deuterium atom at a frequency of 0.5. The enzyme catalyzes no hydrogen exchange at α and β positions of alanine with a solvent deuterium atom. On the other hand, the hydrogen of selenocysteine is fully retained at the α position of alanine. Thus, a two-base mechanism is plausible for the enzyme reaction: the α protonation and deprotonation is performed by one base, and the other base mediates the β protonation.

Selenocysteine β-lyase is inactivated through transamination between selenocysteine and the bound pyridoxal-P to produce pyridoxamine 5'-phosphate (pyridoxamine-P) and a keto analogue of selenocysteine, when the enzyme is incubated with L-selenocysteine in the absence of added pyridoxal-P.[7] The analogous transamination catalyzed by pyridoxal-P enzymes have been reported: serine hydroxymethyltransferase, arginine racemase, tryptophan synthase, aspartate β-decarboxylase, and kynureninase. All of the three enzymes inherently catalyzing β-elimination (kynureninase, aspartate β-

decarboxylase, and selenocysteine β-lyase) catalyze the transamination, though very slowly.

OCCURRENCE OF D-SELENOCYSTINE α,β-LYASE

We found a novel enzyme that catalyzes α,β-elimination of D-selenocystine to produce pyruvate, ammonia and elemental selenium in *Clostridium sticklandii* cells, and named it D-selenocystine α,β-lyase.[8] The enzyme occurs also in *C. sporogenes* but not in other *Clostridium* species. The enzyme was purified to homogeneity from *C. sticklandii*. It has a molecular weight of about 74,000, and consists of two subunits identical in molecular weight (35,000).[8] It requires PLP as a coenzyme.

In addition to D-selenocystine, D-cystine, D-lanthionine, meso-lanthionine, and D-cysteine serve as substrates, but D-selenocysteine is inert. The enzyme catalyzes also the β-replacement reaction between D-selenocystine and various thiols to yield the corresponding *S*-substituted D-cysteines.[8] The relative activities of thiols are summarized in Table II. The rate of α,β-elimination in the presence of

Table II **Relative rate**s of the β-replacement reactioncatalyzed by D-selenocystine α,β-lyase.

Substituent acceptor	Substituent donor	Product	Relative rate (%)
D-Selenocystine	Ethanethiol	*S*-Ethyl-D-cysteine	100
D-Selenocystine	α-Toluenethiol	*S*-Benzyl-D-cysteine	380
D-Selenocystine	2-Hydroxyethanethiol	*S*-(β-Hydroxyethyl)-D-cysteine	2,950
D-Cystine	L-Selenohomocysteine	Selenocystathionine	29

ethanethiol was determined to be only 12% of that of the β-replacement reaction; the β-replacement reaction proceeds preferentially in the presence of *S*-substituent donors. However, the rate of β-replacement reaction was substantially the same as that of α,β-elimination determined in the absence of *S*-substituent donors. In addition, selenols also

can serve as a substrate in the β-replacement reaction, and *Se*-substituted D-selenocysteines corresponding to the selenols used are produced (Table II).

A Schiff base of D-selenocystine is first produced and then converted to a quinoid intermediate through the α-proton abstraction by a base at the active site. The quinoid intermediate undergoes a release of the β-substituent, D-selenocysteine selenide, to generate an α,β-unsaturated intermediate, which is the key intermediate in the α,β-elimination and β-replacement reactions. D-Selenocysteine selenide is labile and is probably converted spontaneously into D-selenocystine and perselenide by disproportionation. Perselenide is also dismutated spontaneously into Se^{2-} and elemental selenium. The α-aminoacrylate produced tautomerizes to α-iminopropionate, which is spontaneously hydrolyzed to form pyruvate and NH_3. When a thiol or selenol attacks the α,β-unsaturated intermediate by a Michael addition, the corresponding *S*-substituted D-cysteine or *Se*-substituted D-selenocysteine is produced.

SYNTHESIS OF METALLOSELENONEIN AND ITS PROPERTIES

Metallothioneins are cysteine-rich proteins with low molecular weights, which bind various kinds of metal ions.[9] The biological functions of metallothioneins have been proposed to be involved in provision of physiological metals for metalloenzymes, and storage and detoxification of heavy metal. These functions depend on their high affinity to metal ions and the reactivity of cysteine residues in a metal-thiolate cluster. We synthesized metalloselenonein, in which all the cysteine residues in the *Neurospora crassa* copper metallothionein are replaced by selenocysteines by solid-phase method with an automated peptide synthesizer.[10] Metalloselenonein has the following sequence:

H-GLY-ASP-SECYS-GLY-SECYS-SER-GLY-ALA-SER-SER-SECYS-ASN-SECYS-GLY-SER-GLY-SECYS-SER-SECYS-SER-ASN-SECYS-GLY-SER-LYS-OH,

where SECYS means selenocysteine. The N^{α}-amino terminus and the side chains of amino acids were protected with acid-labile groups: N^{α}-amino terminus, *t*-butyloxycarbonyl group; aspartic acid and serine, benzyl group. However, no masking group for the selenol of selenocysteine has been developed. We used a *p*-methylbenzyl group to protect the thiol of cysteine. Several new techniques have been developed to reduce side reactions during the peptide synthesis and were used in the present synthesis. For example, Pam resin, which has acid-stable linkage to the resin support, was used to reduce loss of premature peptides during acidolytic deprotection cycles. We used

symmetrical anhydride coupling in dimethylfomamide for 20 min to obtain satisfactory coupling yields of more than 99% for all coupling steps except for the step of Ala8-Ser9 synthesis: only 96.7% coupling yields were achieved.

Metalloselenonein synthesized as described above was purified by preparative HPLC after reduction with $NaBH_4$. The final preparation of metalloselenonein was eluted as a single symmetrical peak upon analytical HPLC, and was found to be homogeneous by amino acid analysis of the acid hydrolysates. The over-all recovery in the preparative HPLC was about 2%. The low yield is probably attributable to the irreversible adsorption of metalloselenonein to the HPLC column. Selenocysteine is oxidized to give selenocysteic acid by performic acid oxidation in the same manner as cysteine. However, selenocysteic acid is decomposed during acid hydrolysis of proteins. Therefore, we analyzed selenocysteine in the form of *Se*-carboxymethyl selenocysteine after alkylation with iodoacetic acid.

Selenium compounds are generally highly susceptible to oxidative degradation. However, we found that the selenol of metalloselenonein is oxidized much more slowly than that of selenocysteine. When metalloselenonein (0.4 mM) was incubated in 0.1 M Tris-HCl buffer (pH 8.0) at 37°C for 2 h, all of the initial selenocysteine residues remained intact, whereas 52% of free selenocysteine was oxidized to selenocystine under the same conditions.

The metalloselenonein complex with Cu(I) was isolated by HPLC with an Asahipak GS-220 gel filtration column. The first major fraction (*Mr* about 2,700) contained 3 gram atoms of copper per mol. The absorption spectrum of the copper complex was characterized by a broad absorption band between 230 and 400 nm with a shoulder around 260 nm. However, metalloselenonein itself showed no absorption above 260 nm. Therefore, the broad absorption band around 280 nm observed for the copper complex is most probably attributed to a copper-selenolate complex. The circular dichroism of the copper-metalloselenonein complex revealed a negative band at 220 nm and a positive band at 245 nm. Metalloselenonein itself showed only a negative band at 220 nm, which is attributable to an amide transition. Therefore, the positive band at 245 nm shows asymmetry in the copper-selenolate coordination. The copper-metalloselenonein complex showed an emission spectrum with a maximum at 395 nm when it was excited at 245 nm. Addition of 1 M HCl to the solution containing the complex led to complete disappearance of not only the absorption band around 250 nm but also the fluorescence band at 395 nm. This indicates displacement of copper with protons in the complex upon addition of HCl. Furthermore, both electron-spectral bands were lost when the complex was oxidized in air.

The Cu-metalloselenonein in complex showed a broad absorption band between 230 and 400 nm with a shoulder around 260 nm. This is probably attributable to Se*-

Cu(I) transition in the copper-selenolate complex. The circular dichroism spectrum of the Cu-metalloselenonein complex showed a positive CD band around 245 nm. This indicates asymmetry in the copper-selenolate complex because the CD band is absent in free metalloselenonein. The fluorescence spectrum of the copper-metalloselenonein complex is also attributable to transitions of a charge-transfer type of the Cu(I)-selenolate complex. Metalloselenonein binds 3 copper ions per mol in contrast to the native and reconstituted metallothionein of *N. crassa* which binds 6 copper ions per mol. In the copper complex of metallothionein, copper atoms are contained in a compact polynuclear cluster with the thiolate of the cysteine residues. The difference in ionic radius between sulfur and selenium most likely accounts for the observed difference in the coordination mode between the two copper-complexes.

EXAFS measurements were carried out for aquaous solutions of selenocysteine and selenocystine, and for selenocystine powdesolidified with polyethylene. The X-

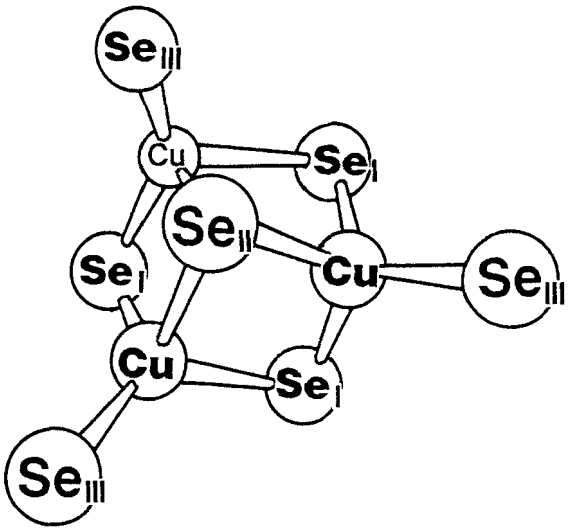

FIGURE 1 Proposed structure of Cu-selenolate cluster in Cu-metalloselenonein.

ray absorption spectrum in the region of the Se K-edge for aquaous solution of L-selenocystine was essentially identical to that for L-selenocystine powder solidified with polyethylene. The bond lengthd were determined as follows: C-Se, 1.88 Å; and Se-Se, 2.32 Å. When L-selenocystine was dissolved in 0.1 M HCl and analyzed, the bond lengths were not influenced by dissociation of COOH and NH_2.

Based on these results, copper EXAFS of Cu-metalloselenonein were measured by fluorescence mode at 18K. The XANES oscillation of Cu-metalloselenonein showed a notable similarity to that of Cu-metallothionein. This indicates that the structure of Cu(II)-thiolate cluster in the Cu-metallothionein is similar to that of Cu(II)-selenoate

cluster in the selenium counterpart. CuS is identical with CuSe in the mode of coordination: Cu is in a tetrahedral coordination to 3 S_I (R= 2.09 Å) and 1 S_{II} (R= 2.32 Å). We have applied these values to the analysis of the structure of Cu-metallothionein complex. FIGURE 1 shows a proposed structure of the Cu(II)-thiolate cluster. We analyzed the structure of Cu-metalloselenonein by the same way: 3 Cu ions are coordinated to 7 selenate anions to form a single cluster. Selenium EXAFS of Cu-metalloselenonein also confirmed this structure (TABLE III).

TABLE III Interatomic distance (Å) of Cu-Se, C-Se, Cu-Cu, Se-Se, and Cu-S bonds of Cu-metallothionein and Cu-metalloselenonein complexes.

	Cu EXAFS	Se EXAFS
$Cu\text{-}Se_I$	2.31	2.29
$Cu\text{-}Se_{II}$	2.36	2.29
$Cu\text{-}Se_{III}$	2.58	2.63
C-Se		1.99
Cu-Cu	3.14	
Se-Se		3.09
$Cu\text{-}S_I$, $Cu\text{-}S_{II}$	2.21	
$Cu\text{-}S_{III}$	2.32	

SYNTHESIS OF GLUTASELENONE AND ITS PROPERTIES

We have synthesized four diastereoisomers of glutaselenone by liquid phase method: H-γ-L-Glu-L-Secys-Gly, H-γ-L-Glu-D-Secys-Gly, H-γ-D-Glu-L-Secys-Gly, and H-γ-D-Glu-D-Secys-Gly, where Secys means selenocysteine. The N^{α}-amino terminus and the side chain of amino acids were protected with acid-labile groups: N^{α}-amino terminus, *t*-butyloxycarbonyl and *p*-methoxybenzyloxy carbonyl group; glutamic acid and glycine, benzyl group. We have attempted to protect the selenol group of selenocysteine with *p*-methoxybenzyl group. Benzyl group has been used to protect the selenol group of selenocysteine in the peptide synthesis. *Se*-Benzyl group is generally removed by the reduction with sodium in liquid ammonia, but side reactions occur. In contrast, *p*-methoxybenzyl group, which we used for protection of the selenol group of

selenocysteine, was easily removed by acidolysis with trimethylsilyl bromosilane containing *m*-cresol and thioanisole in trifluoroacetic acid. Thus, *p*-methoxybenzyl group can serve as a new protecting group for selenol in peptide synthesis, and be used for synthesis of various selenocysteine-containing peptides.

Several new techniques recently developed to reduce side reaction during the deprotection were also used in the present synthesis. For example, 1 M trimethylsilyl bromosilane containing *m*-cresol and thioanisole in trifluoroacetic acid was used as a deprotecting reagent . This procedure is more efficient than the usual method with trifluoromethane sulfonate-trifluoroacetic acid. In order to avoid possible alkylation of the selenol of selenocysteine residue during the trifluoroacetic acid treatment, anisole (14% by volume) was added to trap *p*-methoxybenzyloxycarbonyl cation produced from the removal of N^{α}-*p*-methoxybenzyloxycarbonyl group.

The final preparation of glutaselenone was eluted as a single symmetrical peak upon analytical HPLC, and was found to be homogeneous by amino acid analysis of the acid hydrolysates. The average overall yield of the deprotection reaction was about 10% based on the protected glutaselenone.

The oxidized form of glutaselenone showed a broad absorption band between 270 and 400 nm with a shoulder around 300 nm. An extinction coefficient of glutaselenone at 300 nm was calculated to be about 240. The oxidized form of glutaselenone showed four cotton bands between 210 and 400 nm: 215 (LL isomer(+), LD(+), DL(-), DD(-)), 232 (LL(-), DD(+)), 275 (LL(+), DD(-)), 330(LL(-), DD(+)) nm, which can be derived from asymmetric carbon atoms of glutaselenone.

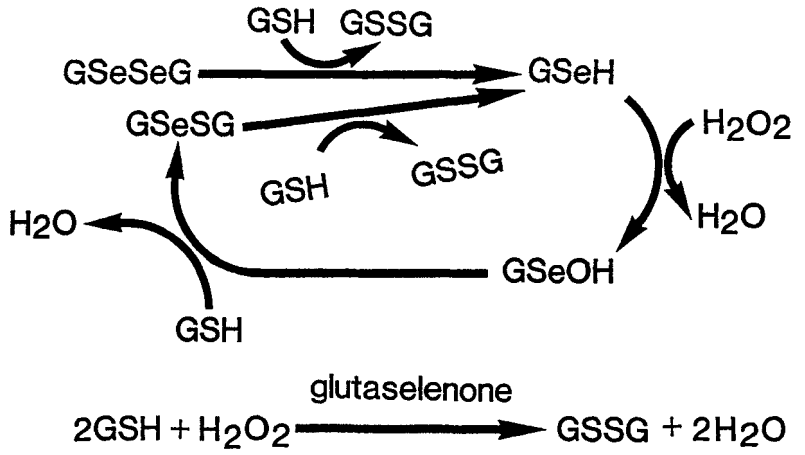

FIGURE 2 Proposed mechanism of glutathione peroxidase activity of glutaselenone.

All the glutaselenone diastereomers showed glutathione peroxidase activity. The activity of the LL-isomer was the highest, which was about 1/4,000 of that of the homogeneous rat lung enzyme, and the LL-isomer was followed by the DL, LD and DD-isomers: the ratio of activities of the LL, DL, LD and DD-isomers was 0.97:0.42:0.11:0.07 for various hydroperoxides. H_2O_2 was better as a substrate than organic hydroperoxides: the relative rate of cumene hydroperoxide and t-butyl hydroperoxide were 48 and 22%, respectively. Glutaselenone showed much higher affinity for glutathione than glutathione peroxidase. The K_m values for glutathione were: the bovine erythrocyte enzyme, 3.0×10^{-3} M; LL-isomer, 8.8×10^{-5} M; DL- isomer, 2.3×10^{-4} M; LD-isomer, 2.3×10^{-4} M; DD-isomer, 5.4×10^{-4} M. However, the affinity for H_2O_2 of glutaselenone was about 10 times lower than that of the bovine erythrocyte enzyme (1.0×10^{-5} M): LL-isomer, 1.0×10^{-4} M; DL-isomer, 1.1×10^{-4} M; LD-isomer, 1.2×10^{-4} M; DD-isomer, 2.8×10^{-4} M. The results show that glutaselenone, in particular the LL-isomer, is a catalyst functioning efficiently. We have isolated the reaction intermediates, and proposed the reaction mechanism. Glutaselenone is first oxidized with hydroperoxides to form glutaselenone selenenic acid, which is reduced with glutathione to regenerate glutaselenone through glutathione-glutaselenone selenosulfide (FIGURE 2). The difference in reactivity among glutaselenone diastereomers is probably based on the rate of formation of the reaction intermediate such as glutathione-glutaselenone selenosulfide.

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