Biochemistry of Selenium Amino Acids

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SUMMARY

Selenocysteine can be synthesized from selenohomocysteine and serine through selenocystathionine by coupling of cystathionine β-synthase and cystathionine γ-lyase reactions. The amount of selenocysteine formed with a rat liver homogenate is exceedingly lower than the value expected from the enzyme activities. The discrepancy is due to degradation of selenocysteine by a novel enzyme in rat liver. The enzyme, termed selenocysteine β-lyase, decomposes specifically L-selenocysteine to L-alanine and elemental selenium. The enzyme occurs in various mammalian and fish tissues, and bacterial, but does not in yeasts, fungi, and plants. The reaction mechanisms of the enzymes purified from pig liver and *Citrobacter freundii* are discussed.

INTRODUCTION

Selenium is now recognized as an essential micronutrient for mammals, birds, fish and several bacteria. 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. Sevebral microbial enzymes also have been demonstrated to contain selenocysteine residue in their polypeptide chains: the selenocysteine residue plays an integral role in the catalysis. Various other natural selenium amino acids occur in a free state. They have been reported to give protection to mammals from radiation injury and carcinogenesis. We here describe enzymological aspects of selenium-containing amino acid metabolism.

I. Synthesis of selenocysteine

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 seleninm compounds have been considered. The indiscriminate catalytic action of enzymes on sulfur and selenuim compounds probably is concerned at least partly in the toxicity of selenium compounds. Provided the selenium compounds are present at high concentrations, indiscriminate substitution of selenium for sulfur in proteins, nucleic acid, and complex carbohydrates could occur and cause various toxic effects on organisms.

Selenomethionine has been demonstrated in wheat and some other grains, but there are few reports of the occurrence of selenocysteine and selenocystine. 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. Evidence for the enzymatic synthesis of selenocysteine was reported by Esaki *et al.*[1] Cystathionine β-synthase of rat liver catalyzes the selenocystathionine formation (equation 1) in addition to the cystathionine synthesis (equation 2).

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 Vmax 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 *Ki* values are substantially consistent with the *Km* 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. Easki *et al.*[1] have found that the α,β-elimination of L-cystathionine (equation 3) proceeds much more slowly (<3%) than the α,γ-elimination (equation 4). However, 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,β-elimination of selenocystathionine proceeds at a comparable rate with the α,β-elimination of the same substrate. Cystathionine γ-lyase can eliminate further L-selenohomocysteine, L-selenocysteine, L-homocysteine, and L-cysteine formed from selenocystathionine and cystathionine by elimination reactions, though slowly. All the selenuim 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

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

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

synthesized directly from L-serine and H_2Se (equation 8).[1] This is probably due to low reactivity of selenide as a substituent donor in replacement reaction as demonstrated with *O*-acetylserine (thiol)-lyase.

II. 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[1]. However, the selenocysteine synthesis proceeds far less efficiently with the homogenate than with the purified enzymes. Easki *et al.*[2] 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. 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).[2]

Selenocysteine β-lyase is distributed widely in mammalian tissues.[2] 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.* However, no significant activity was found in yeasts and fungi.[3]

Selenocysteine β-lyase has been purified to homogeneity from pig liver and *Citrobacter freundii* and characterized[2,4]. The bacterial enzyme is different remarkably 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 pyriodxal 5'-phosphate (pyridoxal-P) as a coenzyme, exhibit strict specificity for L-selenocysteine, and similar *Km* values for the substrates. L-Cysteine behaves as a competitive inhibitor against L-selenocysteine for both the enzymes. Based on the *Km* value for L-selenocysteine and the *Ki* 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 *Km* 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 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.

III. 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. Easki *et al.*[5] and Chocat *et al.*[4] have proposed mechanism of the reactions catalyzed by selenocysteine β-lyase. The selenohydryl group of selenocysteine is substantially in an anionic form under the physiological conditions because its p*Ka* is 5.28. 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 p*Ka* 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 determined by Esaki *et al.*[5] and Chocat *et al.*[4] indicates that an α-hydrogen release occurs in the enzyme reaction and is nate limiting. The α-hydroden 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.

The selenocysteine β-lyase reactions have been studied in deuterium oxide to show deuterium incorporation into alanine by Esaki *et al.*[5] and Chocat *et al.*[4] The ^{1}H and ^{13}C-NMR spectra of alanine produced indicate the formation of [β-$^{1}H_1$] and [β-$^{1}H_2$] alanines. Therefore, in addition to the incoporation of one deuterium atom into 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 α hydrogen of selenocysteine is fully retained at the α position of alanine. Thus, a two-base mechanism has been proposed 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 as reported by Esaki *et al.*[5] and Chocat *et al.*[4] 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.

Chocat *et al.*[4] have reported that selenocysteine β-lyase catalyzes the α,β-elimination of β-chloro-L-alanine to form NH_3, pyruvate, and Cl^{-1}, and is irreversibly inactivated during the reaction in a suicide fashion. The relatively low partition ratio, 825 of the α,β-elimination to the inactivation is similar to that for alanine racemase, and indicates the highly efficient inactivation.

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