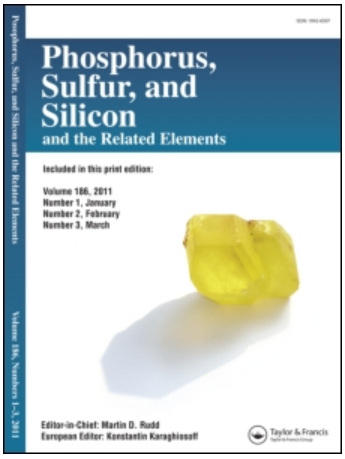

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SYNTHESIS OF BIOLOGICALLY ACTIVE SELENIUM-CONTAINING AMINO ACIDS AND PEPTIDES

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Abstract We here describe the synthesis of selenium amino acids with O-acetylhomoserine sulfhydrylase, partially purified from baker's yeast. The enzyme was found to catalyze the syntheses of L-selenocystine and L-selenohomocystine from sodium diselenide with the corresponding acetyl-derivatives of serine and homoserine, respectively. L-Serine O-sulfate also serves as a substrate of the β-replacement reaction. Sodium diselenide is less efficient as a substituent donor than the physiological substrate, sodium sulfide and inhibits the enzyme at high concentrations. Therefore, limited amounts of sodium diselenide were added to the reaction mixture to increase the yield (about 60%). This provides a facile method to produce optically active selenocystine and selenohomocystine. In addition, we developed a convenient method for the synthesis of a new selenium-containing amino acid, L-selenodjenkolic acid (3,3'-methylenediselenobis(2-amino-propionic acid)) from L-selenocystine thus prepared. This amino acid undergoes α,β-elimination to produce pyruvate, formaldehyde, ammonia and selenium by bacterial methionine γ-lyase under aerobic conditions.

INTRODUCTION

Studies of the metabolism of selenium amino acids have been hampered by difficulties in their syntheses. A variety of chemical syntheses have been described, but most do not directly yield the diselenides (e.g. selenocystine and selenohomocystine), but rather the alkyl or aryl derivatives, in somewhat low yield.[1] These methods are not suitable for biological studies when synthesis of very small amounts of compounds such as optically active

labelled amino acids is required.

We reported previously synthetic procedures giving optically active Se-substituted selenocysteines and selenohomocysteines using bacterial methionine γ-lyase (EC 4.4.1.11)[2] and tryptophan synthase (EC 4.2.1.20).[3] These enzymes could not be used in the direct synthesis of selenohomocystine and selenocystine.

O-Acetylhomoserine sulfhydrylase (O-acetylhomoserine(thiol)-lyase, EC 4.2.99.10)) is a pyridoxal 5'-phosphate enzyme that catalyzes the synthesis of cysteine and homocysteine from H_2S with L-O-acetylserine (OAS) and L-O-acetylhomoserine (OAH), respectively. The enzyme of baker's yeast has been purified and characterized.[4] It is involved in the synthesis *in vivo* of cysteine.

We here describe the synthesis of L-selenocystine and L-selenohomocystine from OAS, L-serine O-sulfate (SOS), or OAH and Na_2Se_2 by the β- and γ-replacement reactions catalyzed by this enzyme, and a convenient method for the preparation of a new selenium-containing amino acid, L-selenodjenkolate (3,3'-methylenediselenobis(2-aminopropionic acid)) from L-selenocystine thus prepared.

ENZYMATIC SYNTHESIS OF SELENOHOMOCYSTINE

When OAH and Na_2Se_2 were incubated at 37°C for 20 min with OAH sulfhydrylase, which was purified partially from baker's yeast according to the method of Yamagata et al,[4] formation of selenohomocystine was confirmed by amino acid analysis (coelution with the authentic compound). The formation of selenohomocystine followed by Michaelis-Menten kinetics up to 10 mM Na_2Se_2. The Km value for Na_2Se_2 was 8.9 mM (Table 1). However, the formation of selenohomocystine was inhibited by higher concentrations of Na_2Se_2. When the enzyme was incubated with various concentrations of

Table 1. Kinetic Parameters of Reactions Catalyzed by OAH Sulfhydrylase

Substituent acceptor	Substituent donor	Rel. Vmax	Km(mM) Substituent	
			Acceptor	Donor
OAH	NaHS	100	4.1	0.52
OAH	Na_2Se_2	17	5.25	8.9
OAS	NaHS	14	2.5	0.7
OAS	Na_2Se_2	8.4	5.0	n.d.
SOS	NaHS	5.1	4.0	0.7
SOS	Na_2Se_2	1.4	4.0	10
SOS	$NaHSe^2$	1.3	n.d.	1.2

n.d., not determined.

Na_2Se_2 and then assayed for selenohomocystine synthetic activity, there was essentially no change, which means that the inhibition by Na_2Se_2 is reversible. The apparent Ki value was 22 mM. When OAS and SOS were used as substituent acceptors of the β-replacement reaction (see below), similar inhibition was observed, and the apparent Ki value for Na_2Se_2 was calculated to be 23 mM. When Na_2Se_2 was replaced by NaHS, inhibition at higher concentrations also occurred.

The optimum pH for the γ-replacement reaction of OAH and Na_2Se_2 was about 8, close to the value for the reaction with NaHS. However, as OAH is converted easily by O-N shift to N-acetylhomoserine at alkaline pH, the reaction was usually carried out at pH 7.2-7.5. The product amino acid was isolated by chromatography from a large-scale incubation mixture and identified as selenohomocystine by ^{1}H-NMR and mass-spectrometry (yield, 60% based on OAH). When the optical purity was examined by HPLC after derivatization with methyl iodide, the D-isomer could not be detected.

ENZYMATIC SYNTHESIS OF SELENOCYSTINE

The OAH sulfhydrylase also catalyzes the synthesis through β-replacement reaction from OAS and NaHS. We attempted to synthesize selenocystine from OAS with a reaction system in which NaHS was replaced by Na_2Se_2. However, the yield was low under these conditions. This is most probably due to the conversion of OAS to N-acetylserine at neutral or alkaline pH. To get better yields, we searched for an alternative substrate and found that SOS is stable in a wide pH range and is cheaply available in chemical industry. The synthesis of selenocystine from SOS proceeded linearly for a long time (Fig. 1), and the yield increased up to about 80%. The kinetic parameters for the β-replacement reactions are summarized in Table 1. The Km values for SOS in the selenocystine and cysteine syntheses were similar to those for OAS, but the Vmax values for OAS were 3-4 times those for SOS. However, the lower reactivity can be compensated by the greater stability of the substrate.

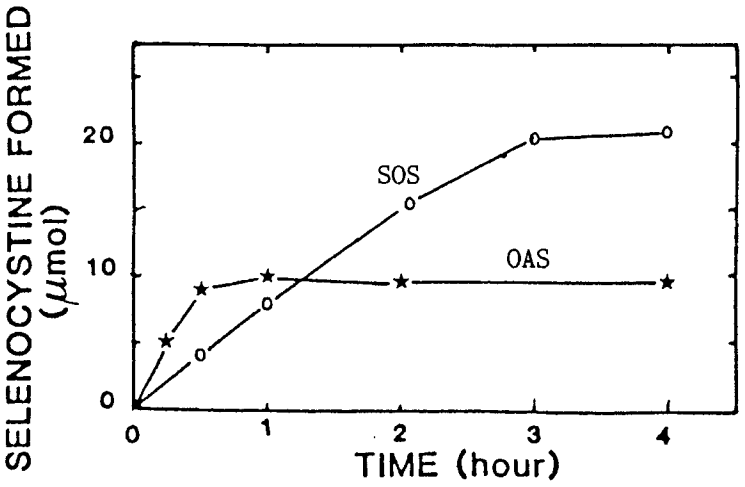

Fig. 1. Enzymatic Synthesis of Selenocystine

SELENODJENKOLIC ACID

Synthesis

L-Selenodjenkolate was prepared from L-selenocystine and methylene iodide as follows. L-Selenocystine (117mg) suspended in 15 ml of H_2O was treated with 1 mmol of $NaBH_4$, followed by addition of 1 mmol of methylene iodide under N_2. After a stirring at room temperature for 2 h, the resulting solution was applied to a Dowex 50-X8 column (H^+, 1 X 40 cm). After washings with water, 2 N HCl, and water, successively, the product was eluted with 4 N NH_4OH, dried under reduced pressure, dissolved in a minimum volume of water, and precipitated by addition of acetone. The compound was crystallized from a small volume of water by addition of acetone (48% yield). The amino acid was identified as L-selenodjenkolate on the basis of several analyses.

Selenium compounds are generally highly susceptible to oxidative degradation. However, the selenodjenkolate synthesized is relatively stable as a selenium compound. When it (2 mM) was incubated in 0.1 M Tris-HCl (pH 8.0) at 37°C for 2 h and analyzed by amino acid analysis, 86% of the initial selenodjenkolate remained intact, whereas only 48% of selenocystine was found to be intact under the same conditions.

Action of Methionine γ-Lyase

When methionine γ-lyase[5] reacted with L-selenodjenkolate, the formation of pyruvate, ammonia, elemental selenium, and formaldehyde was observed. Balance studies showed that 1 mmol of selenodjenkolate is converted into 1 mmol of formaldehyde and 2 mmol each of ammonia, pyruvate, and elemental selenium. A similar result was obtained with sulfur counterparts, L-djenkolate. Kinetic constants were calculated from double-reciprocal plots of

the velocities (as estimated by the pyruvate formation) vs the concentrations of the substrate: L-selenodjenkolate, Km=2.3 mM and Vmax=0.56 unit/mg; L-djenkolate, Km=2.2 mM and Vmax=0.83 unit/mg.

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