Enzymatic synthesis of Se-substituted L-selenocysteine with tryptophan synthase

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1. INTRODUCTION

Selenium is an essential micronutrient for animals and bacteria, and occurs in nature as a constituent of amino acids, nucleosides and other organic compounds [1]. The biological role of selenium amino acids has received considerable attention since selenocysteine (2-amino-3-hydroselenopropionic acid) and selenomethionine (2-amino-4-methylselenobutyric acid) were demonstrated in polypeptide chains of several selenium-containing enzymes (e.g., glutathione peroxidase and glycine reductase) [1–3]. However, a simple synthetic procedure for optically-active selenium amino acids is not available.

Tryptophan synthase (EC 4.2.1.20) is a pyridoxal 5′-phosphate enzyme with multiple catalytic functions [4]. In addition to indole, methanoethiol and 2-mercaptoethanol serve as substituent donors in the β-replacement reactions with L-serine and its derivatives to produce the corresponding Se-substituted L-cysteines [4]. We here describe that Se-substituted L-selenocysteines can be synthesized from L-serine and selenols by the β-replacement reactions catalyzed by tryptophan synthase.

2. MATERIALS AND METHODS

Crystalline α_2–β_2 complex of tryptophan synthase was obtained from *Escherichia coli* (trp R^- Δ trpED102/F′ Δ trpED102) as in [5]. L-Methionine γ-lyase (EC 4.4.1.11) was purified to homogeneity from *Pseudomonas putida* (*Ps. ovalis*) as in [6]. Gaseous methaneselenol was prepared from dimethyldiselenide (Alfa Division-Ventron) as in [7]. α-Tolueneselenol was synthesized as in [8]. Se-Benzyl-L-selenocysteine and Se-methyl-L-selenocysteine were synthesized from β-chloro-L-alanine (Sigma) by the reaction with α-tolueneselenol and methaneselenol, respectively, as in [9].

The β-replacement reactions were performed with the standard reaction mixture containing 6 μmol potassium phosphate buffer (pH 7.8), 3 μmol L-serine, 2 nmol pyridoxal 5′-phosphate, 20 μmol selenol and 0.12 mg crystalline α_2–β_2 complex of tryptophan synthase in a final volume of 0.1 ml in a sealed tube in which air was displaced by N_2. After incubation at 30°C for 10 min, the reaction was terminated by addition of 0.1 ml of 1 N HCl, followed by addition of 0.1 ml of 1 N NaOH. Products were separated by silica gel thin-layer chromatography (TLC) with a solvent system of 1-butanol–acetic acid–water (12:3:5, by vol.), and reacted with ninhydrin at 50°C for 30 min to determine their amounts with a Shimadzu dual-wavelength TLC scanner CS-900. One unit of enzyme was defined as the amount of enzyme that catalyzes the formation of 1 μmol sulfur or selenium amino acid per min. The specific activity of the enzyme is expressed as units per mg protein.

3. RESULTS AND DISCUSSION

When L-serine was incubated with the α_2–β_2

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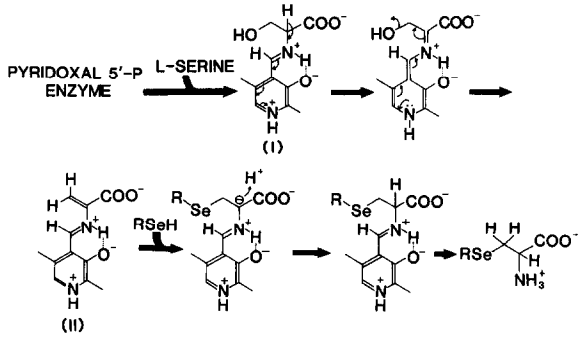
complex of tryptophan synthase in the presence of α-tolueneselenol, the formation of a new amino acid which reacts with both ninhydrin and platinum reagents [10] was observed by TLC. To isolate and identify the amino acid, a large scale reaction mixture (final vol. 5 ml) was incubated at 30°C for 16 h under N_2. No microbial contamination was observed in the reaction mixture during the reaction. After deproteinization by addition of HCl, the supernatant solution was neutralized with NaOH, followed by chromatography on a preparative silica gel chromatoplate (Merck 60 F254, 2 mm thick) with 1-butanol–acetic acid–water (12:3:5, by vol.) as a solvent. The product was eluted with 1 N HCl and applied to a Dowex 50X8 (H^+) column (1 × 5 cm). After the column was washed thoroughly with water, the product was eluted with 1 N HN_4OH, followed by evaporation to dryness. The product crystallized from water was subjected to ^{1}H-NMR analysis with a JEOL JNM-FX 100 spectrometer: at 2.72–3.11 ppm (2H, multiplet, C-3-H_2), 3.62 (2H, singlet, C_6H_5, CH_2-Se-), in deuteriotrifluoroacetic acid with sodium 4,4-dimethyl-4-silapentane-5-sulfonate as an internal standard. The mass spectrum of the product showed characteristic selenium isotope pattern at *m/e* 259 (M^+), 214 (M-45), 186 (M-73) and 172 (M-87), and coincided with that of authentic *Se*-benzylselenocysteine. The main fragment ion at *m/e* 91 was assigned as $C_7H_7^+$. Specific rotation ($[\alpha]_D^{20}$) of the product (1% concentration in 1 N NaOH) was determined to be +38.8° with a Perkin-Elmer recording polarimeter 241. The $[\alpha]_D^{20}$-value of Se-benzyl-L-selenocysteine was reported to be +39.4° [11]. Thus, the product was identified as Se-benzyl-L-selenocysteine. The yield was 44% based on L-serine.

The enzymatic product from methaneselenol was isolated essentially as described above except that gaseous methaneselenol was substituted for α-tolueneselenol. The ^{1}H-NMR spectrum of the product crystallized from ethanol was identified with authentic Se-methylselenocysteine: at 1.93 ppm (3H, singlet, CH_3Se-), 2.76–3.15 (2H, multiplet, C-3-H_2), and 3.88 (1H, quartet, C-2-H) in deuterium oxide with 4,4-dimethyl-4-silapentane-5-sulfonate as an internal standard. The product was α,β-eliminated quantitatively by L-methionine γ-lyase (EC 4.4.1.11), which acts exclusively on the L-isomer [12], but was not oxidized by D-amino acid oxidase of pig kidney (EC 1.4.3.3) (Sigma). These results indicate that the product is Se-methyl-L-selenocysteine. The yield was 16% based on L-serine.

The reactivities of selenols were compared to those of thiols in a reaction system in which L-serine was used as a substrate. Specific activities of the enzyme in β-replacement reaction with α-tolueneselenol and methaneselenol were 0.96 and 0.77, respectively, wherease those with α-toluenethiol and methanethiol were 3.2 and 0.61, respectively. We have identified the products from these thiols as the corresponding Se-substituted L-cysteines by ^{1}H-NMR. The pH optimum of the enzyme in the β-replacement reaction was about 8.0, which is close to the value for the reactions with thiols.

Tryptophan synthase catalyzes β-replacement reaction between the hydroxyl group of L-serine and a selenol to form the corresponding Se-substituted L-selenocysteine as described above. According to the general mechanism for the β-replacement reaction catalyzed by pyridoxal 5′-phosphate enzymes [13,14], nucleophilic addition of selenols occurs in the α-aminoacrylate pyridoxamine 5′-phosphate intermediate (II) derived from the pyridoxal 5′-phosphate aldimine intermediate of L-serine (I, scheme 1). Although selenols are more nucleophilic than thiols, selenols are less reactive substituent donors than thiols in the enzymatic β- and γ-replacement reactions [7,15]. This is compatible with the reactivities of α-tolueneselenol and α-toluenethiol, although methaneselenol is a slightly more efficient substi-

Scheme 1.



Mechanism of β-replacement reaction between L-serine and a selenol catalyzed by tryptophan synthase.

tuent donor than methanethiol. Some physicochemical properties of methaneselenol and methanethiol such as volatility and solubility may affect their reactivities in the enzyme reaction, although further studies are needed to clarify the mechanism.

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