Enzymatic Conversion of *S*-(β -*N*-Acetyl-aminoethyl)-L-cysteine into Its α -Keto Analogue

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SAEC* (thialysine) acts as a metabolic antagonist against L-lysine to depress growth of certain microorganisms.^{1~3)} We reported that *Aerobacter aerogenes* is capable of utilizing SAEC as a sole nitrogen source to accumulate SAcAEC* as the direct metabolite.⁴⁾ The occurrence of an enzyme in the cell-free extract of *A. aerogenes* which catalyzes transfer of acetyl group from acetyl-CoA was subsequently shown,⁵⁾ and the enzyme, acetyl-CoA: SAEC ω -*N*-acetyltransferase, has been purified to homogeneity to characterize it enzymologically.⁶⁾ We investigated also effect of SAEC on incorporation of lysine into protein and the activity of lysyl-tRNA synthetase.⁷⁾ The experiments reported here are concerned with the further metabolism of SAcAEC by *A. aerogenes*.

The resting cells and cell-free extract of *A. aerogenes* were prepared as described previously.^{5,6)} When [³⁵S]-SAcAEC, which was synthesized from [³⁵S]-SAEC essentially according to the procedure of synthesis of ϵ -*N*-acetyl-lysine,⁸⁾ was incubated with resting cells or a crude enzyme, oxygen uptake was observed (Fig. 1). The reaction mixture was then centrifuged at 17,000 *g* at 4°C for 20 min. The supernatant was examined by high-voltage paper electrophoresis in 0.25 M pyridine-acetate buffer (pH 4.8). In addition to SAcAEC, two unknown radioactive compounds were demonstrated (Fig. 2). These compounds were

FIG. 1. Enzymatic Oxidation of SAcAEC and Other Amino Acids.

The enzyme activity was followed by measuring the rate of oxygen uptake with a Warburg manometer. The main compartment of a Warburg vessel contained 100 μ moles of Tris-HCl buffer (pH 7.0) and crude enzyme (4.3 mg protein) in a final volume of 1.9 ml. The center well contained 0.2 ml of 20% KOH. The side arm contained 10 μ moles of SAcAEC or certain other L-amino acids in 0.4 ml. Measurements were made at 30°C in the air. A blank in which substrate was replaced by water was run for every assays of the enzyme.

FIG. 2. Conversion of SAcAEC into Compounds A and B.

The reaction mixture contained 50 μ moles of [³⁵S]-SAcAEC, 1 mmole of Tris-HCl buffer (pH 7.5) and the resting cells (8.01 mg dry weight) in a final volume of 9.0 ml. After incubation at 30°C for 60 min, the reaction was terminated by addition of 1.0 ml of 50% trichloroacetic acid. The clear supernatant (20 μ l) obtained by centrifugation was subjected to paper electrophoresis at 3000 V for 20 min. The radioactivity was followed by a paper chromatogram scanner (Aloka, JPC-102).

termed tentatively compounds A and B in the order of their increasing mobilities. Both compounds were not visualized with ninhydrin but with a platinum reagent. Only compound A reacted with 2,4-dinitrophenyl-hydrazine

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* Abbreviations: SAEC, *S*-(β -aminoethyl)-L-cysteine; SAcAEC, *S*-(β -acetyl-aminoethyl)-L-cysteine.

(0.5% in 2 N HCl) to form the hydrazone. When the reaction was carried out in the presence of semicarbazide (100 μ moles), the radioactivity was found exclusively in the semicarbazone of compound A. These findings suggest that SAcAEC is converted into compound A, and compound B successively, and that A is a carbonyl compound. Thus, attempts were made to isolate and identify compound A.

The reaction mixture containing [35 S]-SAcAEC and a crude enzyme was incubated as described above and deproteinized. The supernatant was applied to a column of Dowex-50 $\times$ 8 (H $^{+}$ form, 3.5 $\times$ 70 cm) to adsorb the unreactive substrate, and eluted thoroughly with water. The eluate, which contained compounds A and B, was concentrated at 40°C under reduced pressure, and chromatographed on a Dowex-1 $\times$ 8 column (acetate form, 3.5 $\times$ 70 cm). Aliquots of each fraction were used to determine the radioactivity with a Tri-Carb Scintillation Counter 3320. Compounds A and B were eluted with 1.0 N and 0.5 N acetic acid, respectively. To the solution of compound A was added 2 ml of 0.5% 2,4-dinitrophenylhydrazine solution dissolved in 2 N HCl to yield the hydrazone. To examine the possibility that compound A may be a keto analogue of SAcAEC, *S*-(β -*N*-acetyl-aminoethyl)- α -ketomercaptopropionate was prepared by enzymatic oxidation of SAcAEC using L-amino acid oxidase according to a modification of the procedure of Meister⁹⁾ as follows. After dialysis catalase (100 units) and L-amino acid oxidase (100 mg) from snake venom (Sigma Biochemical Co., U.S.A.) were added successively to a suspension (pH 7.4) of 1 g of SAcAEC in 50 ml of water. After oxygen was bubbled through the suspension for 12 hr, the mixture was filtered with a Dia flow filter (Amicon, U.S.A.). The filtrate was extracted with ethyl acetate under acidic conditions. The ethyl acetate fractions were evaporated to dryness at 45°C. The residue was crystallized and recrystallized from acetone-ether; mp 184~185°C (decomposition), (Found: C, 41.51; H, 5.43; N, 6.78%. Calcd. for C₇H₁₁O₄-

NS: C, 40.96; H, 5.40; N, 6.82%). The solutions of compound A and synthetic *S*-(β -*N*-acetyl-aminoethyl)- α -ketomercaptopropionate were subjected to paper chromatography with butane-1-ol-acetic acid-water (4:1:1) as a solvent. The paper developed and dried was sprayed with a solution of 0.05% 2,4-dinitrophenylhydrazine solution in 2 N HCl, and dipped in 2% NaOH solution in 90% ethanol. The *R_f* value of compound A was identical with that of the synthetic compound (*R_f*, 0.84). When the electrophoresis of compound A was carried out in 0.25 M pyridine-acetate buffer (pH 4.8), a spot which was positive to the 2,4-dinitrophenylhydrazine test migrated the same distance as the synthetic compound. The 2,4-dinitrophenylhydrazones of compound A and the synthetic *S*-(β -*N*-acetyl-aminoethyl)- α -ketomercaptopropionate, prepared by treatment with 2,4-dinitrophenylhydrazine in the usual way, when chromatographed on paper using butane-1-ol-ethanol-water (4:1:4, upper layer) gave two identical spots (*R_f*, 0.32 and 0.42). It is likely that the 2,4-dinitrophenylhydrazone exists in *syn*- and *anti*-forms due to the isomerism around the double bond. Thus, compound A was identified as *S*-(β -*N*-acetyl-aminoethyl)- α -ketomercaptopropionate, an α -keto analogue of SAcAEC. The findings suggest that SAcAEC was oxidatively deaminated to form the α -keto analogue by an oxidase. L-Amino acid oxidase has been demonstrated in *Neurospora crassa*, *Aspergillus niger*, *Proteus vulgaris* and *Aerobacter aerogenes* as reviewed by Meister.¹⁰⁾ Monoamino-monocarboxylic acids are known to be most susceptible to the L-amino acid oxidases. L-Methionine and L-leucine also served as good substrates in the enzyme system, whereas L-lysine and SAEC, which are very poor substrates of common L-amino acid oxidase, were oxidized only slightly as shown in Fig. 1. These results indicate that after ω -*N*-acetylation SAEC is metabolized to *S*-(β -*N*-acetyl-aminoethyl)- α -ketomercaptopropionate by the action of L-amino acid oxidase in *A. aerogenes* as follows.

Compound B did not react with ninhydrin and 2,4-dinitrophenylhydrazine, but did with a platinum reagent, and moved toward the anode electrophoretically. Therefore, compound B is probably *S*-(β -*N*-acetyl-aminoethyl)- α -hydroxymercaptopropionate or the closely related compound as postulated for lysine metabolism of *Neurospora*¹¹⁾ and *Hansenula saturnus*.¹²⁾

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