ENZYMIC *N*-ACETYLATION OF A SULFUR ANALOG OF L-LYSINE, *S*-(β-AMINOETHYL)-L-CYSTEINE

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1. Introduction

It is known that *S*-(β-aminoethyl)-L-cysteine termed also L-thialysine, inhibits the growth of lactic acid bacteria as a potent antimetabolite of L-lysine [1, 2]. *Aerobacter aerogenes* was found to be capable of growing well in the medium containing this L-lysine antagonist as a sole nitrogen source, and the metabolic product has been isolated and identified as *S*-(β-*N*-acetylaminoethyl)-L-cysteine [3]. This compound was also found as a main product from *S*-(β-aminoethyl)-L-cysteine in the growing cultures of *Neurospora crassa* [4]. In the present work, evidence has been obtained for occurrence of an enzyme in the cell-free extract of *Aerobacter aerogenes*, which catalyzes the transfer of acetyl group from acetyl-CoA to the terminal amino group of *S*-(β-aminoethyl)-L-cysteine.

2. Materials and methods

S-(β-Aminoethyl)-L-cysteine.HCl was synthesized from L-cysteine.HCl (Ajinomoto Co., Tokyo) and ethylenimine by a modification [5] of the method of Cavallini et al. [6]. *S*-(β-Aminoethyl)-L-cysteine-3-^{14}C was also synthesized in the same manner from L-cysteine-3-^{14}C, which was prepared by reduction of L-cystine-3-^{14}C (Dai-ichi Chemicals, Tokyo) with zinc. Acetyl phosphate and acetyl-CoA were prepared according to the procedure of Avison [7], and by the treatment of CoA (Boehringer Mannheim GmbH) with acetic anhydride [8], respectively. Phosphotransacetylase (*Clostridium kluyveri*) was purchased from Boehringer Mannheim GmbH. The specific activity was approximately 1,000 international units per mg of protein.

Aerobacter aerogenes IFO 3320 was grown in the medium containing 0.5% pepton, 0.1% meat extract, 0.1% glucose, 0.1% NaCl, 0.1% KH_2PO_4, 0.1% K_2HPO_4, 0.01% $MgSO_4.7H_2O$, 0.01% *S*-(β-aminoethyl)-L-cysteine and 0.01% yeast extract (pH 7.2). The cultures were grown in a 10-l jar fermentor at 28° for 17 to 20 hr under aeration. The cells harvested by centrifugation were washed twice with 0.85% NaCl solution. The washed cells were suspended in 0.01 M tris-HCl buffer, pH 7.4, containing 0.01% 2-mercaptoethanol, and subjected to sonication in a 19-kc Kaijo Denki oscillator at 0–5° for 15 min. After centrifugation at 17,000 *g* for 30 min, the supernatant was dialyzed against the above buffer, and used as a crude enzyme.

The enzyme catalyzing the transfer of acetyl group was assayed by determination of *S*-(β-*N*-acetyl-aminoethyl)-L-cysteine formed with ninhydrin after separation by paper chromatography as described previously [9]. Protein was determined by the method of Lowry et al. [10].

3. Results and discussion

When *S*-(β-aminoethyl)-L-cysteine was incubated with acetyl phosphate at pH 7.8 in the presence of washed cells or crude enzyme, *S*-(β-*N*-acetyl-aminoethyl)-L-cysteine was formed and identified as described previously [3]. The bacterial and enzymic incorporation of radioactivity of *S*-(β-aminoethyl)-L-cysteine-3-^{14}C into *S*-(β-*N*-acetyl-aminoethyl)-L-

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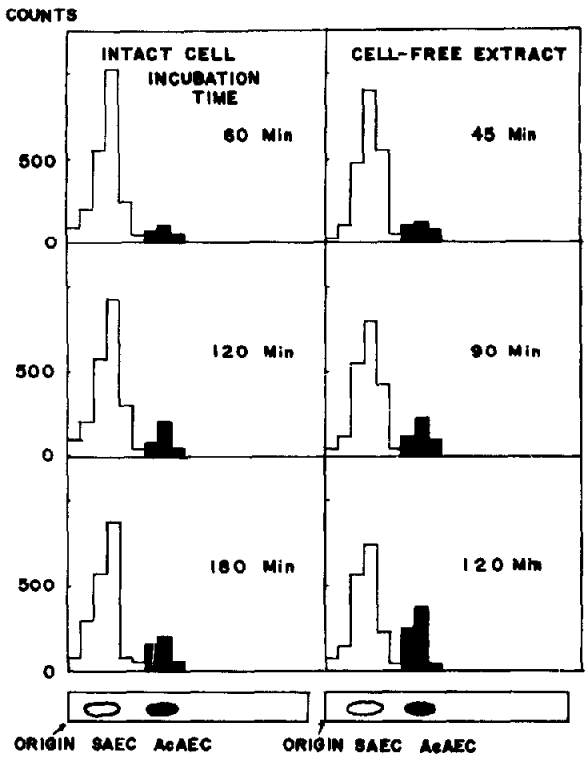

Fig. 1. Incorporation of the radioactivity into *S*-(β-*N*-acetyl-aminoethyl)-L-cysteine from *S*-(β-aminoethyl)-L-cysteine. The reaction mixture contained 10 μmoles of *S*-(β-aminoethyl)-L-cysteine, 0.025 μmole of *S*-(β-aminoethyl)-L-cysteine-3-^{14}C, 20 μmoles of acetyl phosphate, 1 μmole of CoA, 500 μmoles of potassium phosphate buffer, pH 7.4, and the intact cells (6.03 mg dry weight) or the crude enzyme (1.2 mg protein) in a final volume of 0.9 mL After incubation at 37° for various periods, the reaction was terminated by addition of 0.1 ml of 50% trichloroacetic acid. The clear supernatant (20 μl) obtained by centrifugation was chromatographed on Toyo No. 53 filter paper, using ethanol, ammonia and water (18:1:1) as a solvent. The paper strip (3 X 40 cm) was cut into serial 1.5-cm sections. They were transferred to scintillation vials containing the toluene-dioxane-ethylcellosolve system, and the radioactivity was measured with a Tri-Carb Liquid Scintillation 3320. SAEC: *S*-(β-aminoethyl)-L-cysteine, AcAEC: *S*-(β-*N*-acetyl-aminoethyl)-L-cysteine.

cysteine was investigated. Two radioactive spots corresponding to the starting material and its *N*-acetylated product are seen (fig. 1). The radioactivity of *S*-(β-*N*-acetyl-aminoethyl)-L-cysteine increased and that of the substrate decreased as the incubation was prolonged in both the reaction systems. It is clear

Table 1
Acetyl donor specificity

Acetyl donor	Formation of AcAEC (μmoles) *	
	I	II
Sodium acetate	0	0
Acetyl phosphate	4.37	1.98
Acetyl phosphate + CoA	9.54	4.82
Acetyl phosphate + CoA **	10.02	5.92
Acetyl-CoA	9.38	9.40
Acetyl phosphate ***	0.93	0.93

* The reaction mixture contained 20 μmoles of *S*-(β-aminoethyl)-L-cysteine, 20 μmoles of acetyl donor with or without 1 μmole of CoA, 500 μmoles of tris-HCl (I) or potassium phosphate (II) buffer, pH 7.4, and 2 mg of enzyme protein in a final volume of 0.9 ml. Incubation was carried out at 37° for 2 hr.
** Phosphotransacetylase (2 μg) was added.
*** The enzyme was omitted.

that *S*-(β-*N*-acetyl-aminoethyl)-L-cysteine is formed as the exclusive product from the substrate.

Attempts were made to ellucidate the acetyl donor (table 1). The possibility that *S*-(β-aminoethyl)-L-cysteine is acetylated enzymically in the presence of sodium acetate, as in the case of enzymic synthesis of *N*-acetyl amino acids by acylase I [11], was excluded. Acetyl-CoA and acetyl phosphate were shown to be effective acetyl donors in tris-HCl buffer, but the latter required the presence of CoA for the full activity. When the incubation was carried out in the presence of potassium phosphate buffer, which inhibited the phosphotransacetylase activity approximately 40%, *N*-acetylation was diminished in the reaction systems containing either acetyl phosphate alone or acetyl phosphate and CoA, although acetyl-CoA was utilized as well as in tris-HCl buffer. This fact suggests that acetyl-CoA is the direct donor of acetyl group. The non-enzymic *N*-acetylation with acetyl phosphate was observed to a certain extent under the conditions employed. This enzyme preparation contained considerably high activity of phosphotransacetylase, because its addition to the reaction mixture resulted in only the slight activation. The findings described above present good evidence for occurrence of an enzyme catalyzing the transfer of acetyl group from acetyl-CoA to the terminal amino

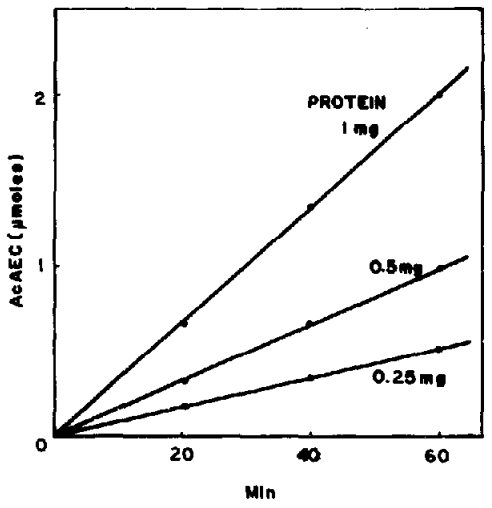

Fig. 2. Relationship between the rate of synthesis of *S*-(β-*N*-acetyl-aminoethyl)-L-cysteine, and the incubation time and the enzyme concentration. The reaction mixture consisted of 5 μmoles of *S*-(β-aminoethyl)-L-cysteine, 10 μmoles of acetyl phosphate, 0.5 μmole of CoA, 2 μg of phosphotransacetylase, 500 μmoles of tris-HCl buffer, pH 7.8, and enzyme in a final volume of 0.9 ml. The enzyme was omitted in a blank. Incubation was carried out as described in fig. 1.

group of *S*-(β-aminoethyl)-L-cysteine, (acetyl-CoA:*S*-(β-aminoethyl)-L-cysteine ε-*N*-acetyltransferase).

$$NH_2.CH_2.CH_2.S.CH_2.CH(NH_2).COOH + CH_3.CO\text{-}CoA$$

$$\rightarrow CH_3.CO.NH.CH_2.CH_2.S.CH_2.CH(NH_2).COOH + CoA.$$

The enzymic acetylation with acetyl phosphate alone implies that the crude enzyme preparation must contain a small amount of CoA.

The rate of the enzymic synthesis of *S*-(β-*N*-acetyl-aminoethyl)-L-cysteine is directly proportional to the incubation time and the enzyme concentration as shown in fig. 2.

Further work on the purification and properties of the enzyme is under investigation.

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