Effect of S-(β-Aminoethyl)-L-Cysteine on Incorporation of Lysine into Protein in Bacteria

Author(s)
Tanaka, Hidehiko; Soda, Kenji

Citation

Issue Date
1974-12-25

URL
http://hdl.handle.net/2433/76568

Type
Departmental Bulletin Paper

Textversion
publisher

Kyoto University
Effect of S-(\(\beta\)-Aminoethyl)-L-Cysteine on Incorporation of Lysine into Protein in Bacteria

Hidehiko Tanaka* and Kenji Soda*

Received July 26, 1974

S-(\(\beta\)-Aminoethyl)-L-cysteine, a sulfur analogue of lysine inhibited strongly growth of E. coli A-19, and weakly that of Corynebacterium sp. isolated from soil, but did not inhibit growth of A. aerogenes. In C. sp. the inhibitory effect was markedly enhanced in the presence of L-threonine. The inhibition of growth by S-(\(\beta\)-aminoethyl)-L-cysteine was rapidly reversed by the addition of L-lysine.

S-(\(\beta\)-Aminoethyl)-L-cysteine inhibited protein synthesis and the activity of lysyl-tRNA synthetase from E. coli and A. aerogenes. All the other lysine analogues tested inhibited the activity of enzyme, but S-(\(\beta\)-aminoethyl)-L-cysteine derivatives, S-(\(\beta\)-N-acetyl-aminoethyl)-L-cysteine and S-(\(\beta\)-aminoethyl)-\(\alpha\)-N-acetyl-L-cysteine were not effective.

INTRODUCTION

The structural analogues of naturally occurring amino acids have proven of interest in the biochemical and therapeutical aspects. The studies on the action of amino acid analogues and microbial mutants resistant to them have served to shed light on the mechanism of metabolic regulation. Some of amino acid analogues function as a false corepressor or a false feedback inhibitor of enzymes participating in biosynthesis of the corresponding natural amino acids, and also inhibit incorporation of the amino acids into protein. But, certain microorganisms are not only insusceptible to the amino acid analogues, but also capable of utilizing them.

Some lysine analogues show metabolic antagonism as reviewed by Meister. A sulfur analogue of lysine, S-(\(\beta\)-aminoethyl)-L-cysteine was reported to inhibit growth of Leuconostoc mesenteroides, Lactobacillus arabinosus and Brevibacterium flavum. We, however, showed that Aerobacter aerogenes is capable of utilizing S-(\(\beta\)-aminoethyl)-L-cysteine as a sole nitrogen source, and that the main metabolic product isolated is S-(\(\beta\)-N-acetyl-aminoethyl)-L-cysteine. The occurrence of an enzyme in the cell-free extract of A. aerogenes which catalyzes transfer of acetyl group from acetyl-CoA to the terminal amino group of S-(\(\beta\)-aminoethyl)-L-cysteine, and properties of the purified enzyme were also reported. The present paper describes the effect of this lysine analogue on protein biosynthesis.

EXPERIMENTAL PROCEDURES

Bacteria and Growth Conditions

The following bacterial strains were used; Escherichia coli A-19 (RNase I-, Met-),
Effect of \( \text{S-(\(\beta\)-Aminoethyl)} \)-L-Cysteine on Protein Biosynthesis

\textit{A. aerogenes} IFO 3320, and the wild strain and the \( \text{S-(\(\beta\)-aminoethyl)} \)-L-cysteine resistant mutant of \textit{Corynebacterium} sp., which was isolated from soil. The organisms were grown on M9 medium, \( 10^3 \) g of peptone, 15 g of \( \text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} \), 3 g of \( \text{KH}_2\text{PO}_4 \), 1 g of \( \text{NH}_4\text{Cl} \), 1 ml of 1 M \( \text{MgSO}_4 \), 10 ml of 40% glucose and 1000 ml of distilled water. The cultures were carried out with 500 ml of the medium placed in a 2000-ml flask on a reciprocating shaker at 28°.

\textbf{Chemicals}

\textit{S-(\(\beta\)-Aminoethyl)}-L-cysteine-HCl was synthesized from L-cysteine and ethylenimine by a modification of the method of Cavallini \textit{et al.}\textsuperscript{14) S-(\(\beta\)-Aminoethyl)}-L-[\(^{35}\text{S}\)]-cysteine was also synthesized in the same manner from L-[\(^{35}\text{S}\)]-cysteine (specific activity; 53 mc/m-mole, the Radiochemical Center, England). The other lysine analogues, O-(\(\beta\)-aminoethyl)-DL-serine, 2-amino-3-(2-aminoethylamino)-propionic acid, lysine hydroxamate and \( \text{S-(\(\beta\)-aminoethyl)} \)-L-cysteine hydroxamate were prepared as described previously.\textsuperscript{11) Creatine phosphate, creatine phosphate kinase and tRNA were purchased from Boehringer Mannheim GmbH. The other chemicals were analytical grade reagents.

\textbf{Preparation of Ribosome and Amino Acyl tRNA Synthetase.}

According to the procedure of Nirenberg \textit{et al.},\textsuperscript{13) ribosome and amino acyl tRNA synthetase were prepared as follows. Bacterial cells harvested in mid-log. phase were washed with M9 medium and were disrupted by grinding with levigated alumina at 4° in a mortar. To the mortar was added the equivalent weight of 0.01 M Tris-HCl buffer, pH 7.8, containing 0.01 M magnesium acetate, 0.06 M KCl and 0.006 M mercaptoethanol (the standard buffer) and mixed well with paste. The supernatant solution obtained by centrifugation at 20,000 g for 20 min was incubated with DNase (2 \( \mu \text{g} \) per ml) at 4° for 10 min, and then centrifuged again at 20,000 g for 20 min. The supernatant fluid was centrifuged at 30,000 g for 30 min. The liquid layer was aspirated (S-30 fraction), and was ultracentrifuged at 105,000 g for 2 hr. To three-fifth of the supernatant solution (S-100 fraction) aspirated, was added streptomycin sulfate (5%, weight by volume) to precipitate nucleic acids. After 30 min, the precipitate was removed by centrifugation. The supernatant solution was brought to 20% saturation with solid ammonium sulfate, followed by centrifugation. Ammonium sulfate was added to the supernatant to 70% saturation. The precipitate collected by centrifugation was dissolved in the standard buffer, dialyzed against two changes of this buffer overnight, and used as amino acyl tRNA synthetase.

\textbf{Protein Biosynthesis}

The reaction mixture consisted of 25 \( \mu \text{moles} \) of Tris-HCl buffer, pH 7.8, 25 \( \mu \text{moles} \) of KCl, 5 \( \mu \text{moles} \) of magnesium acetate, 1 \( \mu \text{mole} \) of ATP, 5 \( \mu \text{moles} \) of creatine phosphate, 25 \( \mu \)g of creatine phosphate kinase, 0.125 \( \mu \text{mole} \) of GTP, 10 \( \mu \text{g} \) of polyadenylic acid, radioactive amino acid (\( ^{14}\text{C} \)-L-lysine or \( \text{S-(\(\beta\)-aminoethyl)} \)-L-[\(^{35}\text{S}\)]-cysteine) and S-30 fraction in final volume of 0.5 ml. The reaction was initiated by addition of radioactive amino acid. After incubation at 37° for 15 min, the reaction mixture was deproteinized by addition of 5% trichloroacetic acid containing 0.25% sodium tungstate. The deproteinized mixture was placed in a water bath at 90° for 10 min and then was chilled in ice for 30 min. The suspension was filtered under suction through a Millipore filter (HA, 25
mm in diameter, 0.45-µ pore size). After two washings with cold 5% trichloroacetic acid containing 0.25% sodium tungstate, the radioactivity on the filter was determined with a scintillation spectrometer.

**L-Lysyl-tRNA Synthetase Activity**

Lysyl-tRNA synthetase was assayed by measuring the incorporation of [14C]-L-lysine into an acid-precipitable fraction. The assay mixture contained in a final volume of 0.4 ml; 50 µmoles of Tris-HCl buffer, pH 7.4, 5 µmoles of magnesium chloride, 5 µmoles of KCl, 1 µmole of ATP, 1 µmole of 2-mercaptoethanol, 0.5 mg of *E. coli* MRE 600 tRNA, 10 µmole of [14C]-L-lysine and S-100 fraction. The reaction was initiated by addition of [14C]-L-lysine. After incubation at 37° for 5 and 10 min, 50-µl portions were removed to filter paper discs (diameter, 25 mm), and the discs were immediately immersed in cold 10% trichloroacetic acid. After one washing with cold 10% trichloroacetic acid, two washings with cold 5% trichloroacetic acid, and one washing with acetone, the discs were transferred to scintillation vials containing the toluene system. Radioactivity was measured with a Tri-Carb liquid scintillation spectrometer 3320.

**RESULTS**

**Effect of S-(β-Aminoethyl)-L-Cysteine on the Growth of Bacteria**

*E. coli* A-19, *A. aerogenes*, and the wild strain and S-(β-aminoethyl)-L-cysteine resistant mutant of *Corynebacterium* sp. were used. As shown in Fig. 1, growth of *E. coli* was inhibited completely when 2 mg/ml of S-(β-aminoethyl)-L-cysteine was added to the medium. Growth of *C. sp.* decreased only about 30% even in the presence of high concentration of S-(β-aminoethyl)-L-cysteine (5 mg/ml). But the inhibition of growth of *C. sp.* was markedly enhanced by addition of L-threonine as found for *B. flavum* by Sano et al.9 To ascertain whether this growth inhibition caused by S-(β-aminoethyl)-L-cysteine was related to lysine metabolism, effect of lysine on the inhibition was examined. To the bacterial culture grown in the presence of 2 mg/ml of S-(β-aminoethyl)-L-cysteine was added L-lysine (final concentration: 1.4 mM). The addition of L-lysine recovered fully growth at the concentration of one-tenth of the inhibitor. This finding suggests that the lysine analogue depresses an enzyme related to lysine biosynthesis, or an enzyme system catalyzing the incorporation of L-lysine into the protein to cause inhibition of growth. *A. aerogenes* IFO 332010 and an S-(β-aminoethyl)-L-cysteine resistant mutant grew well even in the presence of 5 mg/ml of S-(β-aminoethyl)-L-cysteine.

**Effect of S-(β-Aminoethyl)-L-Cysteine on Incorporation of L-Lysine into Protein**

Effect of S-(β-aminoethyl)-L-cysteine on the incorporation of L-lysine into protein was examined. S-(β-Aminoethyl)-L-[35S]-cysteine was not incorporated into protein, when the protein biosynthesis system from the above mentioned four bacterial strains, and polyadenylic acid were used. When the S-30 fractions of the four strains were incubated with [14C]-L-lysine, synthesis of polylysine was observed. To the reaction mixture was added 0.4 mM of S-(β-aminoethyl)-L-cysteine, followed by incubation. The incorporation of [14C]-L-lysine was inhibited approximately 60% in the systems of *E. coli* and *A. aerogenes*.
Effect of S-(β-Aminoethyl)-L-Cysteine on Protein Biosynthesis

Fig. 1. Effect of S-(β-aminoethyl)-L-cysteine on growth of bacteria. Cells harvested in the exponential growth phase were inoculated into a minimum medium in test tubes. Turbidity of the medium was measured at the time when the cultures in the unsupplemented control medium showed maximum turbidity.

1: A. aerogenes, 2: E. coli A-19, 3: Corynebacterium sp., 3': Corynebacterium sp. in the presence of L-threonine, whose concentrations were equal to those of S-(β-aminoethyl)-L-cysteine, 4: S-(β-Aminoethyl)-L-cysteine resistant mutant of Corynebacterium sp.

The inhibition of protein synthesis in E. coli and A. aerogenes systems increased with increasing the concentration of S-(β-aminoethyl)-L-cysteine in the reaction mixture. Ten mM S-(β-aminoethyl)-L-cysteine inhibited almost completely incorporation of L-lysine. This concentration is close to concentration that causes inhibition of growth of E. coli. The inhibitory effect on the protein synthesis of A. aerogenes was very similar to that of E. coli. We reported that A. aerogenes is capable of growing well in the medium containing S-(β-aminoethyl)-L-cysteine, and that this lysine analogue is acetylated to yield S-(β-N-acetyl-aminoethyl)-L-cysteine by S-(β-aminoethyl)-L-cysteine ω-N-acetyltransferase of the organism. Incorporation of lysine into protein was also investigated in the presence of several S-(β-aminoethyl)-L-cysteine derivatives. The compounds in which the amino groups of S-(β-aminoethyl)-L-cysteine were blocked, S-(β-aminoethyl)-α-N-acetyl-L-cysteine and S-(β-N-acetyl-aminoethyl)-L-cysteine have only negligible influence on the incorporation (Table II).
Table I. Effect of S-(β-Aminoethyl)-L-Cysteine on Incorporation of L-Lysine

The assay was performed with 200 μM of [14C]-L-lysine and indicated concentration (mM) of S-(β-aminoethyl)-L-cysteine. The other conditions were given in the Experimental Procedures.

<table>
<thead>
<tr>
<th>L-SAEC* (mM)</th>
<th>Inhibition (%)</th>
<th>E. coli</th>
<th>A. aerogenes</th>
<th>C. sp.</th>
<th>C. sp. Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>57</td>
<td>62</td>
<td>12</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>64</td>
<td>71</td>
<td>14</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>89</td>
<td>92</td>
<td>15</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

* L-SAEC: S-(β-aminoethyl)-L-cysteine

Table II. Effect of S-(β-Aminoethyl)-L-Cysteine Derivatives on L-Lysine Incorporation (A. aerogenes)

The conditions were given in Table I.

<table>
<thead>
<tr>
<th>L-SAEC Derivatives (1 mM)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>L-SAEC</td>
<td>71</td>
</tr>
<tr>
<td>L-AcSAEC</td>
<td>3</td>
</tr>
<tr>
<td>AEAC</td>
<td>0</td>
</tr>
<tr>
<td>L-Homo SAEC</td>
<td>58</td>
</tr>
</tbody>
</table>

L-AcSAEC: S-(β-N-acetyl-aminoethyl)-L-cysteine
AEAC: S-(β-aminoethyl)-α-N-acetyl-L-cysteine
L-Homo SAEC: S-(β-aminoethyl)-L-homocysteine

Table III. Effect of Lysine Analogues on L-Lysyl-tRNA Synthetase Activity

The incubation was performed for 10 min. Concentrations of [14C]-L-lysine, O-(β-aminoethyl)-DL-serine and the other lysine analogues were 0.15, 2 and 1 mM, respectively.

<table>
<thead>
<tr>
<th>Lysine Analogues</th>
<th>Inhibition (%)</th>
<th>E. coli</th>
<th>A. aerogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>DL-Oxalysine</td>
<td>70</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>L-Azalysine</td>
<td>76</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>L-Homo SAEC</td>
<td>66</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>L-Lysine Hdx</td>
<td>59</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>L-SAEC Hdx</td>
<td>68</td>
<td>68</td>
<td></td>
</tr>
</tbody>
</table>

DL-Oxalysine: O-(β-aminoethyl)-DL-serine
L-Azalysine: 2-amino-3-(2-aminoethylamino)-propionic acid
Hdx: hydroxamate

(510)
Effect of S-(β-Aminoethyl)-L-Cysteine on Protein Biosynthesis

Fig. 2. Effect of S-(β-aminoethyl)-L-cysteine on the l-lysyl-tRNA synthetase reaction. The assay was performed with 0.5 unit of enzyme from *E. coli* A-19 (A) or *A. aerogenes* (B), 150 μM of lysine, and indicated concentrations (mM) of S-(β-aminoethyl)-L-cysteine.

Table IV. Effect of S-(β-Aminoethyl)-L-Cysteine on the L-Lysyl-tRNA Synthetase Activity

<table>
<thead>
<tr>
<th>Strains</th>
<th>L-SAEC (mM)</th>
<th>Activity* (cpm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>None</td>
<td>1033</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>311</td>
<td>70</td>
</tr>
<tr>
<td><em>A. aerogenes</em></td>
<td>None</td>
<td>938</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>322</td>
<td>66</td>
</tr>
<tr>
<td><em>C. sp.</em></td>
<td>None</td>
<td>1180</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1195</td>
<td>0</td>
</tr>
<tr>
<td><em>C. sp.</em> L-SAEC Resistant</td>
<td>None</td>
<td>1815</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1975</td>
<td>0</td>
</tr>
</tbody>
</table>

* Incubation was carried out for 10 min.

**Effect of S-(β-Aminoethyl)-L-Cysteine on the Lysyl-tRNA Synthetase Reaction**

Effect of S-(β-aminoethyl)-L-cysteine on the lysyl-tRNA synthetase activity was investigated. The activity of lysyl-tRNA synthetase was decreased by the addition of S-(β-aminoethyl)-L-cysteine as shown in Fig. 2. Lysyl-tRNA synthetase activity was determined also in the presence of several other lysine analogues. In addition to S-(β-aminoethyl)-L-cysteine, all of other lysine analogues tested inhibited markedly the enzyme (Table III), while l-lysyl-tRNA synthetase from the wild strain and the S-(β-aminoethyl)-L-cysteine resistant mutant of *C. sp.* was not influenced by S-(β-aminoethyl)-L-cysteine (Table IV).
Several analogues of lysine have been reported to function as metabolic antagonists; for example, 5-hydroxylysine, 2,6-diaminoheptanoic acid, 3-aminomethylcyclohexane glycine, 3-aminocyclohexane alanine and trans-4-dehydrolysine. The replacement of the 4-methylene group of lysine by an oxygen, sulfur or nitrogen atom leads to the potent lysine antagonists; O-(β-aminoethyl)-serine, S-(β-aminoethyl)-cysteine and 2-amino-3-(2-aminoethylamino)-propionic acid. Either O-(β-aminoethyl)-serine or 2-amino-3-(2-aminoethylamino)-propionic acid antagonizes lysine to inhibit growth of *E. coli* and various lactobacilli. The inhibition of growth of *L. dextranicum* by 2-amino-3-(2-aminoethylamino)-propionic acid was competitively reversed by lysine over about 30-fold range of concentrations with an inhibition index of about 10.

Growth of *E. coli* A-19 is strongly inhibited by S-(β-aminoethyl)-L-cysteine, while *A. aerogenes* is capable of utilizing it to grow well. S-(β-Aminoethyl)-L-cysteine alone was weakly inhibitory to growth of *Corynebacterium* sp., but the inhibition was markedly enhanced by the addition of L-threonine. This phenomenon seems to result from a pseudo-concerted feedback inhibition by S-(β-aminoethyl)-L-cysteine and threonine as found in *B. flavum*. Similar concerted feedback inhibition was also found in *Rhodopseudomonas capsulata* and *Bacillus polymyxa*. S-(β-Aminoethyl)-L-cysteine strongly inhibited protein synthesis and the activity of lysyl-tRNA synthetase from *E. coli* and *A. aerogenes*. In addition to S-(β-aminoethyl)-L-cysteine, O-(β-aminoethyl)-DL-serine, 2-amino-3-(2-aminoethylamino)-propionic acid, hydroxamates of L-lysine and S-(β-aminoethyl)-L-homocysteine inhibited the activity of the enzyme. The specificities of aminoacyl-tRNA synthetases with respect to their amino acid substrates have been extensively studied. For example, L-serine hydroxamate was demonstrated to be a competitive inhibitor of seryl-tRNA synthetase from *E. coli* K-12 with a Ki value of 30 µM. Recently, Kisselev et al. reported that lysine analogues; D-lysine, amide and hydrazide of L-lysine, and methyl and ethyl esters of L-lysine inhibit the activity of purified lysyl-tRNA synthetase from *E. coli* B, and that their inhibition is competitive with L-lysine, although effect of 4-substituted lysines was not investigated. Decrease of lysyl-tRNA synthetase activity leads probably to depression of protein synthesis and growth of *E. coli* A-19. Three different forms of aspartokinase are operative in the feedback control of lysine biosynthesis in *E. coli*. S-(β-Aminoethyl)-L-cysteine acts probably on aspartokinase to cause interference in growth. The ability of *A. aerogenes* that is capable of utilizing S-(β-aminoethyl)-L-cysteine is attributable to the action of S-(β-aminoethyl)-L-cysteine ω-N-acetyltransferase, because the N-acetylated analogue does not inhibit the activity of lysyl-tRNA synthetase as shown in Table IV. Lysyl-tRNA synthetase from the C. sp. wild strain and the S-(β-aminoethyl)-L-cysteine resistant mutant was not influenced by S-(β-aminoethyl)-L-cysteine. These findings suggest that the mutant contains aspartokinase which is insensitive to inhibition by S-(β-aminoethyl)-L-cysteine.

**ACKNOWLEDGMENTS**

We thank Dr. M. Takanami for his kind supply of strain *Escherichia coli* A-19, and also Dr. T. Yamamoto for his advice.
Effect of S-(β-Aminoethyl)-L-Cysteine on Protein Biosynthesis

REFERENCES