Note

Effects of a Selenium Analogue of L-Lysine on Growth of *Candida pelliculosa*Eiichi SHIMIZU,^{*,†} Ryutaro YAMANA,^{††}
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Selenium, a homologue of sulfur and tellurium is an essential micronutrient for mammals, fish and several bacteria. The biological role of selenium amino acids has received considerable attention since selenocysteine (α -amino- β -hydroselenopropionic acid) and selenomethionine (α -amino- γ -methylselenobutyric acid) were demonstrated in polypeptide chains of several selenium-containing enzymes.¹⁾ *Se*-(β -Aminoethyl)-L-selenocysteine (L-selenalysine), in which the γ -methylene group of lysine is replaced by a selenium atom, has been reported to act as a competitive inhibitor of lysine in protein synthesizing

systems from *Escherichia coli*, rat liver and rabbit reticulocytes.²⁾ Little attention, however, has been paid to the biological effect of the analogue on yeasts. The corresponding sulfur analogue, *S*-(β -aminoethyl)-L-cysteine (L-thialysine) has been reported to inhibit growth of certain microorganisms as a potent metabolic antagonist against L-lysine.^{3~5)} Recently, we reported that the sulfur analogue acts as a false feedback inhibitor of homocitrate synthase [EC 4.1.3.21] from *Candida pelliculosa*,⁶⁾ which catalyzes the initial step of lysine biosynthesis in yeasts and fungi. We are interested in examining whether the biological effects of L-thialysine are enhanced by substituting a selenium atom for the sulfur analogue. This paper describes the biological effect of L-selenalysine on *C. pelliculosa* in comparison with L-thialysine.

L-Selenalysine·HCl was synthesized from selenocystamine·HCl and β -chloro-L-alanine·HCl according to the method of De Marco *et al.*⁷⁾ L-Thialysine was prepared from ethylenimine and L-cysteine·HCl as described previously.⁸⁾ *C. pelliculosa* (IFO 0707) was cultured in Lingens-Oltmanns minimum medium⁹⁾ supplemented with various concentrations of L-selenalysine. Homocitrate synthase activity was measured by CoA formed as described previously.⁶⁾

L-Selenalysine completely inhibited the growth of *C. pelliculosa* for a certain period, but did not affect the growth rate, as shown in Fig. 1; the organisms grew at a normal rate after a lag period even in the presence of 4 mM L-selenalysine. The initiation of cell growth was delayed with increasing concentration of L-selenalysine in the

FIG. 1. Effect of L-Selenalysine on Growth of *Candida pelliculosa*.

A 0.1 ml aliquot of the seed culture was transferred into 4 ml of the minimum medium supplemented with various concentrations of L-selenalysine (○, none; ▲, 0.4 mM; ■, 1 mM; ●, 2 mM; ▲, 4 mM), and incubated at 28°C on a reciprocating shaker. Cell growth was measured turbidimetrically at 660 nm. The inset shows the relationship between the concentration of L-selenalysine and the lag period.

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medium, and the lag period was proportional to its concentration in the range of 0.4~4 mM (Fig. 1, inset). The time course curves and the extent of the growth inhibition are similar to those for L-thialysine reported previously.⁸⁾

To elucidate whether the inhibition by L-selenalysine was related to lysine metabolism, we examined the effect of L-lysine and the related amino acids. The addition of L-lysine to the culture medium effectively eliminated the growth inhibition by L-selenalysine in proportion to the concentration, though D-lysine was inert; L-lysine at concentrations higher than about 1.4 mM almost fully restored the inhibition in the presence of 4 mM L-selenalysine. The protective effect of L-lysine, however, was lower than that observed with L-thialysine: the inhibition by 4 mM L-thialysine was substantially eliminated by 0.4 mM L-lysine. L- α -Aminoadipate, one of the intermediates in lysine biosynthesis, and L-arginine also restored the inhibition by L-selenalysine, although less effectively. Other amino acids tested were not at all or only slightly effective. The protective effect of L-selenalysine-inhibition by L-lysine and L- α -aminoadipate suggests that, like L-thialysine, L-selenalysine interfered directly with lysine biosynthesis or its utilization. The effect of L-arginine is probably ascribable to its structural similarity to L-lysine.

To confirm whether L-selenalysine interferes with lysine biosynthesis, the effect of the selenium analogue on the activity of homocitrate synthase from *C. pelliculosa* was studied with a reaction system (1.0 ml) containing 100 μ mol of Tris-HCl buffer, pH 8.5 (at this pH the inhibition was most potent), 50 μ mol of potassium α -ketoglutarate, 0.17 μ mol of acetyl-CoA and the enzyme. L-Selenalysine significantly inhibited the enzyme at a concentration over 0.5 mM as a false feedback inhibitor, and at a concentration of 7.5 mM the inhibitory effect was 90%. The concentration of L-selenalysine for half-maximal inhibition was calculated to be 1.9 mM. We have reported previously that the concentrations of L-lysine and L-thialysine for half-maximal inhibition are 0.02 and 1.1 mM, respectively.⁶⁾ Thus, L-selenalysine shows a less marked

inhibitory effect than L-lysine and L-thialysine. De Marco *et al.*²⁾ also reported that compared to L-thialysine, L-selenalysine shows a slightly lower competitive effect against L-lysine in protein synthesizing systems from *E. coli*, rat liver and rabbit reticulocytes.²⁾ We studied the inhibitory effect of both the lysine analogues kinetically. L-Selenalysine showed a competitive inhibition against both acetyl-CoA (K_i , 1.1 mM) and α -ketoglutarate (K_i , 0.14 mM). L-Thialysine also inhibited the enzyme in a competitive manner with acetyl-CoA (K_i , 0.94 mM), but did not affect the binding of α -ketoglutarate to the enzyme. This finding indicates that the action of L-selenalysine on the enzyme is different from that of L-thialysine that binds to the same allosteric site as L-lysine. The detailed mechanism and other biological properties of the selenium lysine analogue are currently under investigation.

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