# ON THE REGULATION OF ASPARAGINASE SYNTHESIS IN *PSEUDOMONAS BOREOPOLIS* 526

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Received 5 October 1972

## 1. Introduction

The antitumor activity of bacterial L-asparaginase and its distribution among various taxonomic groups of microorganisms has been extensively studied [1–4]. The amides of dicarboxylic amino acids probably play an important role in cell metabolism. However, the regulation of L-asparaginase biosynthesis has not been studied extensively and only in a limited number of microorganisms. Biochemical characteristics of different asparaginase-producing microorganisms are very variable so that the data obtained in studies of one microorganism may be inapplicable for another. In a previous paper [5] it was pointed out that the regulation of L-asparaginase synthesis can take place by different mechanisms even in microorganisms of one species, such as *Ps. aurantiaca* 875 and *Ps. boreopolis* 526. For example, in *Ps. aurentiaca* addition of asparagine resulted in induction of enzyme synthesis while in *Ps. boreopolis* 526 it repressed the process. However, one cannot exclude the possibility of an indirect effect of products of asparagine metabolism. This point was investigated and the results are presented below.

## 2. Materials and methods

*Pseudomonas boreopolis* 526 was grown for 18 hr in a synthetic medium with 0.5% of yeast extract. Cells were collected by centrifugation, suspended in fresh medium and incubated for 3 hr at 26°; neutralised solutions of aspartate and asparagine ($2 \times 10^{-3}$ M) were then added. The incubation was continued at 26°; after incubation for 3, 6, 9, 12, 20, 30 and 60 min, aliquots were taken for L-asparaginase and L-glutaminase assay. The bacterial cells were washed twice with cold water and then with Tris-HCl buffer, pH 7.4 and subjected to toluene treatment. Cell-free extract was obtained by centrifugation of the disrupted cells and used as an enzyme source. In control experiments, cells were grown in the same medium, to which instead of aspartate and asparagine corresponding amounts of ammonium sulfate and glycerol were added.

## 3. Results and discussion

Fig. 1 shows data on the effect of L-asparagine on asparaginase synthesis in *Ps. boreopolis* 526. Asparagine increased the enzyme synthesis in this organism by 20–25% within 3 min after addition of the substrate. The increase in the enzyme formation was only temporary and within 12–20 min the asparaginase activity of the cells subsided to control levels or even lower. During further growth of the cells in the presence of asparagine, a progressive decrease in the enzyme activity was noted whereas this activity in the control cells increased. Aspartate added to the medium during the first minutes did not cause considerable change in L-asparaginase activity of the cells, but in 3–6 min after addition of this substance, some temporary increase in this activity could be easily observed (fig. 2). Highest values of enzyme activity in the cells were recorded within 9–12 min; on further incubation there was a gradual decrease in this activity.



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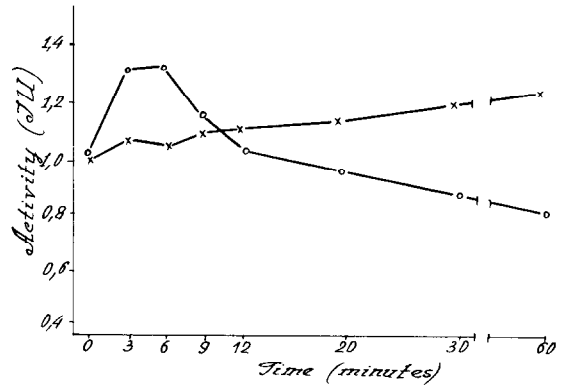


Fig. 1. Effect of L-asparagine ($2 \times 10^{-3}$ M) on L-asparaginase synthesis in *Ps. boreopolis* 526. *The enzyme activity was determined as described previously [4]. (o—o—o) Asparagine; (x—x—x) control.

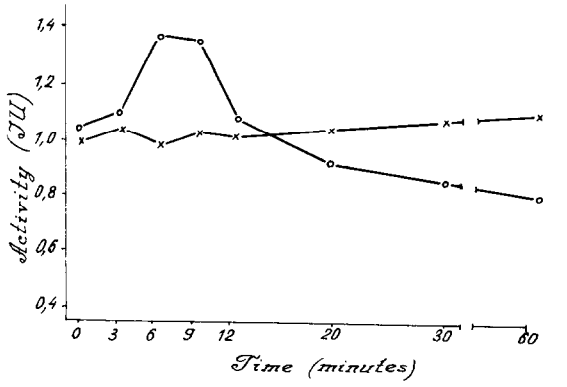


Fig. 2. Effect of L-aspartate ($2 \times 10^{-3}$ M) on L-asparaginase synthesis in *Ps. boreopolis* 526. (o—o—o) Aspartate; (x—x—x) control.

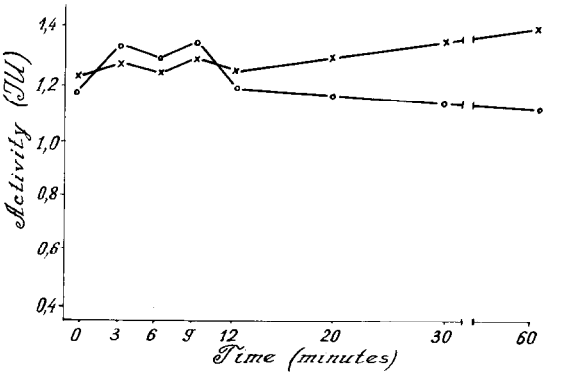


Fig. 3. Effect of L-aspartate ($2 \times 10^{-3}$ M) on L-glutaminase synthesis in *Ps. borepolis* 526. (o—o—o) Aspartate; (x—x—x) control.

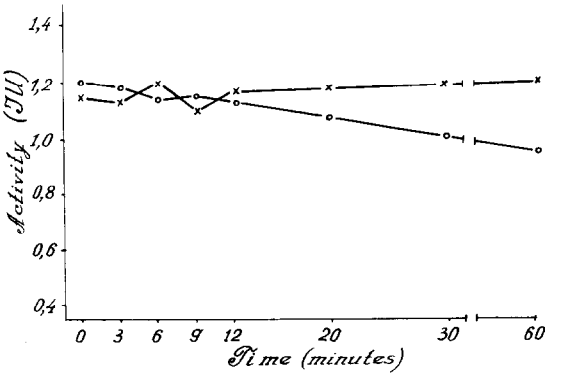


Fig. 4. Effect of L-asparagine ($2 \times 10^{-3}$ M) on L-glutaminase synthesis in *Ps. boreopolis* 526. (o—o—o) Asparagine; (x—x—x) control.

The shape of L-asparaginase activity curves obtained in the medium with aspartate and asparagine (fig. 1, 2) seems to suggest that there is a double control mechanism regulating enzyme synthesis in *Ps. boreopolis* 526. Asparaginase synthesis is induced by the substrate of the reaction (fig. 1). This conclusion was indirectly confirmed by the enzyme activity curve obtained with aspartate (fig. 2). A transient increase in enzyme activity 6 min after adding asparate could be explained by the synthesis of asparagine in the cells. At the same time L-asparaginase synthesis was repressed by the reaction product (or products).

The regulation system was apparently more sensitive to repression. Gradual decrease in the enzyme activity after transient but considerable increase confirms this conclusion. The influence of L-aspartate upon enzyme synthesis was probably an indirect effect, because within the first 3–6 min after addition of this substance, L-asparaginase activity in the cells remained at the same level as in control cells; after an increase in the enzyme activity, gradual decrease was noted. Repression of enzyme synthesis was apparently due to the effect of aspartate metabolites since aspartate itself did not change the level of asparaginase synthesis. Previous work from this laboratory [5] has shown that the cell-free extract of *Ps. boreopolis* could also hydrolyse L-glutamine (110–120% of L-asparaginase activity level). It was shown in further experiments that in the medium with aspartate and

with asparagine the L-asparaginase activity curves were quite similar in shape to those for L-glutaminase activity in the presence of glutamate and glutamine. We made an attempt to separate these activities by ammonium sulfate and ethanol fractionation and also by gel filtration on Sephadex G-100 and Sephadex G-200. Our results suggest that the enzyme hydrolyses both amides, but the possibility of existence of two amide-hydrolyzing enzymes with close physical and chemical properties cannot be excluded.

The L-glutaminase activity curves (figs. 3,4) presented in this communication show that during the first 12 min L-asparagine and L-aspartate do not influence considerably the L-glutaminase activity under our experimental conditions.

These data suggest that L-asparaginase and L-glutaminase activities in *Ps. boreopolis* 526 might involve two separate amidases having similar physical and chemical properties. In this particular case, L-asparagine and L-glutamine would be the true agents triggering L-asparaginase and L-glutaminase synthesis, respectively, while products of their metabolism could act as repressors of the enzyme synthesis.

## References

[1] R.E. Handschumacher and J. Osumi, Proc. Am. Assoc. Cancer Res. 11 (1970) 34.
[2] V.A. Zanin, T.T. Berezov, A.Ya. Nikolaiev and A.H. Abdumalikov, Mikrobiologia 38 (1969) 3.
[3] V.V. Eremenko and E.S. Tulpanova, Prikl. Biokhimia Mikrobiologia 5 (1971) 7.
[4] R.K. Ozolin and E.Z. Ruban, Nauchn. Dokl. Vissch. Shk. Biol. Nauki 6 (1971) 110.
[5] V.A. Zanin, T.T. Berezov and A.Ya. Nikolaiev, Izv. A.N. SSSR, Ser. Biol. 3 (1972) 338.