CHARACTER OF ASPARAGINASE AND GLUTAMINASE SYNTHESIS IN Pseudomonas boreopolis 526

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Experiments with Pseudomonas boreopolis 526 showed that L-asparagine and L-glutamine are the most probable inducers of synthesis of L-asparaginase and L-glutaminase, respectively, while their metabolic products are repressors of the synthesis of the deaminases. The presence of asparagine synthetase in the investigated strain and the inducing action of L-aspartate on the formation of this enzyme also were demonstrated.

Investigations by Soviet biochemists have shown [2, 4] that asparagine and glutamine play a wider role than was hitherto recognized [8] in the metabolic reactions of living organisms. In recent years enzymes catalyzing the deamination of these amides have come to be used as therapeutic substances against tumors in animals and man [1, 9, 10] for both glutamine and asparagine have been shown to be essential factors for growth of certain types of tumors in tissue culture [7]. Positive results have been obtained in the treatment of leukemias by asparaginase and glutaminase, although not in all cases, and this has necessitated a method of obtaining sufficient quantities of these enzymes for clinical use. In the attempt to solve this problem it is important to know the conditions of cultivation of the producer microorganisms which are optimal for the production of the enzymes as well as the mechanism for the regulation of their biosynthesis [3, 5, 6].

The object of this investigation was to study the effect of asparagine, glutamine, and the corresponding dicarboxylic amino acids on the synthesis of asparaginase and glutaminase in Pseudomonas boreopolis 526.

EXPERIMENTAL METHOD

Strain Ps. boreopolis 526 grown on synthetic medium with the addition of 0.05% yeast extract was used. The cells were collected by centrifugation, transfered to fresh medium, and grown for a further 3 h, after which the test substance was added in a concentration of 2×10^{-3}M. Aspartate and glutamate were added after neutralization. The cell suspension in the medium with the added substances was kept on a shaker (120 rpm) at 26°C. Samples were withdrawn 3, 6, 9, 12, 20, 30, and 60 min after the additions were made in order to determine the activity of L-asparaginase and L-glutaminase, the source of which was the protein extract obtained after destruction of the washed cells.

In the continuous cultivation experiments cells grown in synthetic medium in a flask for 18 h were transfered with sterile precautions into a cultivator (volume 160 ml, rate of flow 18-20 ml/h, aeration 0.5 liter/min). After cultivation of the cells for 24 h on basic medium the medium with the test substance was collected. Each subsequent substance was added after the passage of 5-6 volumes of basic medium through the cultivator. The cells were collected every 2 h and the deaminase activity determined.

To determine asparagine synthetase samples (1 ml) containing 20 μmole NH_4Cl, 5 μmole $MgCl_2$, 3 μmole ATP, 4 μmole glutathione, 30 μmole tris-HCl buffer (pH 7.6), 0.2-0.3 mg protein, and 4 μCi DL-as-

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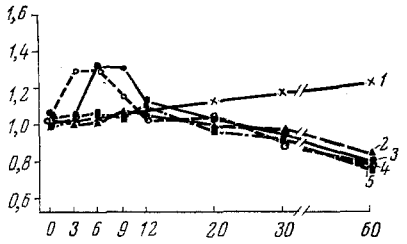

Fig. 1

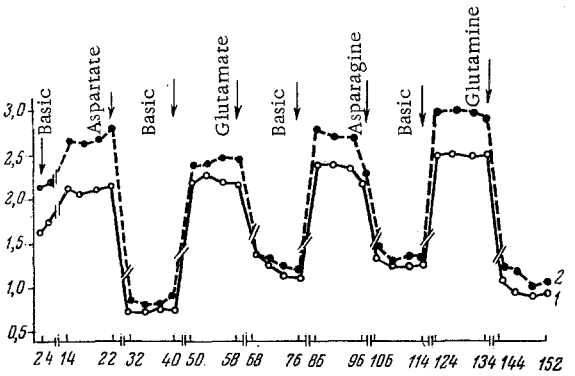

Fig. 2

Fig. 1. Effect of L-aspartate, glutamate, asparagine, and glutamine (2×10^{-3}M of each) on asparaginase synthesis by Ps. boreopolis 526. Abscissa, time (in min); ordinate, activity (in i.u.): 1) control; 2) glutamate; 3) aspartate; 4) asparagine; 5) glutamine.

Fig. 2. Effect of aspartate, glutamate, asparagine, and glutamine on synthesis of L-asparaginase (1) and L-glutaminase (2) by Ps. boreopolis 526 during continuous cultivation. Abscissa, time of cultivation (in h); ordinate, activity (in i.u.). Arrow – time of change of medium.

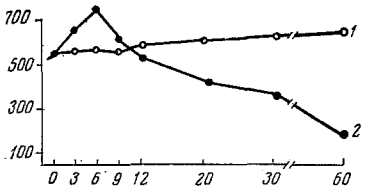

Fig. 3. Effect of aspartate (2×10^{-3}M) on formation of L-asparagine synthetase by Ps. boreopolis 526. Abscissa, time (in min); ordinate, activity (pulses [min] mg protein): 1) control, 2) aspartate.

partate were incubated for 30 min at 37°C. The reaction was stopped by the addition of 0.5 ml 1 N $HClO_4$; the pH of the medium was adjusted to 5.7 by the addition of 7 N KOH and the residue was removed by centrifugation. An aliquot of the samples was applied to a column with alumina (0.7×10 cm), and the C^{14} asparagine formed was eluted from the column by 10 ml H_2O. The quantity of labeled asparagine in the eluate was measured on a scintillation counter. Asparagine synthetase activity was expressed in pulses [min] mg protein.

EXPERIMENTAL RESULTS AND DISCUSSION

It will be clear from Fig. 1 that the addition of asparagine led within 3 min to an increase of 25-30% in L-asparaginase activity in Ps. boreopolis. However, this increase was of short duration, and 12-20 min after addition of the substrate the asparaginase activity was normal again. During continued cultivation of the cells with asparagine (up to 60 min) a decrease in activity of the enzyme was observed, whereas in the control cells this activity increased gradually. In the presence of aspartic acid the asparaginase activity during the first minutes was the same as in the control, but it increased after 3-6 min and reached a maximum after 9-12 min, after which it fell.

Glutaminase activity of the cells was not significantly different from the control in the presence of asparagine and aspartate for the first 20 min, but like the asparaginase activity it progressively decreased during further incubation in the presence of glutamine and glutamate. During incubation of the cells with glutamine and glutamate, on the other hand, the glutaminase activity increased while the asparaginase activity showed no significant change during the first 20 min. Further growth of the cells (up to 60 min) on media with these additions led to an appreciable decrease in both activities (Fig. 1).

Repression of deaminase synthesis by dicarboxylic amino acids and their amides was most demonstrative when these substances were added during continuous cultivation (Fig. 2). Under those conditions deaminase synthesis on the basic medium (with a limiting concentration of glycerol) was three to four times more intensive than on medium with additives.

The results of a study of the formation of asparagine synthetase during incubation of the cells with L-aspartate are illustrated in Fig. 3. The asparagine synthetase activity of the cells was increased 3 min after addition of the substrate; however, further incubation of the cells led to sharp inhibition of this activity and, in general, the course of the curve of asparagine synthetase activity repeated the course of the curve of asparaginase activity during incubation of the cells with aspartate.

Regulation of enzyme synthesis in Ps. boreopolis 526 is evidently under the control of a double mechanism. On the one hand synthesis of the enzyme is induced by the reaction substrate (Fig. 1), while on the other hand it is repressed by its metabolite (metabolites).

This conclusion may be indirectly confirmed by the course of the curve of asparaginase activity in the presence of aspartate (Fig. 2); the short increase in activity of the enzyme in this case can be explained by the synthesis of asparagine in the cell. This conclusion assumes the presence of asparagine synthetase, synthesizing asparagine from aspartate in sufficient quantities to induce asparaginase. Special experiments showed that cells of Ps. boreopolis 526 possess asparagine synthetase (Fig. 3); addition of aspartate to the growth medium induces the synthesis of this enzyme. These results demonstrate that the short increase in asparaginase activity of the cells on the addition of aspartate is connected with the preliminary synthesis of asparagine. A similar conclusion is evidently valid also for L-glutaminase during growth of the cells in medium with glutamate.

LITERATURE CITED

1. T. T. Berezov, Vestn. Akad. Med. Nauk SSSR, No. 11, 35 (1971).
2. A. E. Braunshtein, Vestn. Akad. Med. Nauk SSSR, No. 5, 45 (1959).
3. V. A. Zanin, T. T. Berezov, and A. Ya. Nikolaev, Izvest. Akad. Nauk SSSR, Seriya Biol., 3, 338 (1972).
4. S. R. Mardashev, Uspekhi Sovr. Biol., 40, 8 (1955).
5. S. R. Mardashev, N. N. Sokolov, L. P. Evseev, et al., Biokhimiya, No. 3, 529 (1969).
6. H. Cedar and J. Schwartz, J. Bact., 96, 2043 (1968).
7. I. Levintow, Science, 126, 611 (1957).
8. A. Meister, Biochemistry of the Amino Acids, New York (1957).
9. M. Hadhbin, C. Tan, L. Tallal, et al., Proc. Amer. Ass. Cancer Res., 11, 33 (1970).
10. J. Roberts, J. S. Holcenberg, and W. C. Dolowy, J. Biol. Chem., 247, 84 (1971).