REPRESSION OF PROTEASE IN BACILLUS MEGATERIUM BY SINGLE AMINO ACID

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The formation of proteolytic enzymes by bacilli /Chaloupka and Křečková, 1962; Neumark and Citri, 1962/, as well as by actinomycetes /Chaloupka, 1956/, is inhibited in the presence of protein hydrolysate or mixture of amino acids in the medium. It is possible to repress the synthesis of the enzyme in B.cereus by simultaneous addition of high concentrations of threonine and histidine to the basic mixture of amino acids necessary for the growth of the bacillus /Neumark and Citri, 1962/. Results presented in this communication show that the protease repression can be achieved by addition of single specific amino acid to the synthetic salt + glucose medium.

As in our previous work the strain KM of Bacillus megaterium growing on the synthetic C/G medium /McQuillen, 1955/ containing amonium salt as the only source of nitrogen was used in our experiments. The culture grown overnight on the solid medium was washed with 0.5% solution of NaCl and intensively aerated during 2 hours in the liquid medium. Under these conditions the number of cells was usually increased by 50-70%. The growing cells were then resuspended in the fresh medium to the density corresponding to 0.25-0.30 mg dry weight/ml and

1×10^{-3}M $CaCl_2$ was added to stabilize the protease. The 12 ml aliquotes of the suspension were pipetted into Erlenmeyer flasks and amino acids up to concentration of 2×10^{-3}M /DL amino acids/ or 1×10^{-3}M /L amino acids/ were added. The flasks were incubated on a shaker for 1 hour at 35°C. After incubation 9 ml sample was harvested, chloramphenicol /100 ug/ml/ was added, and proteolytic activity was determined /Chaloupka and Křečková, 1962; Chaloupka, Liebster and Janeček, 1958/. 1 uC/ml of ^{35}S methionine was added to the rest of suspension and the mixture was further incubated for 10 min. on the shaker. The same volume of 10% TCA was then added to the suspension and the radioactivity was determined in hot TCA insoluble fraction. According to the influence of single amino acids on the protease formation it is possible to divide them into three groups:

1. Aminoacids repressing the enzyme formation by less than 30%: alanine, arginine, aspartic acid, cysteine, phenylalanine, glycine, glutamic acid, histidine, lysine, methionine, proline, serine, and tryptophan.
2. Amino acids inhibiting by 30-60%: leucine and valine.
3. Amino acids causing repression by more than 60%: isoleucine and threonine.

Amino acids of the second group /leucine and valine/ are inhibitory in the given concentration for the incorporation of ^{35}S methionine approximately to the same extent as for the protease formation and, therefore, their influence on the enzyme synthesis may not be probably specific. On the other hand, the amino acids isoleucine and threonine causing considerable repression of protease did not inhibit at all the incorporation of labeled methionine into the proteins. They did not influence the activity of the protease as well. The influence

of different concentrations of these amino acids on the enzyme formation is presented in the table.

TABLE I

Concentration	DL-threonine Inhibition in %	DL-isoleucine Inhibition in %
5×10^{-3} M	85.1 ± 2.8	---
2×10^{-3} M	79.0 ± 3.6	75.6 ± 9.0
1×10^{-3} M	58.4 ± 7.3	63.5 ± 12.1
5×10^{-4} M	38.9 ± 7.7	52.9 ± 10.8

The values represent an average calculated from 7 experiments in the case of threonine and from 4 experiments in the case of isoleucine.

It is obvious that either amino acid can inhibit the protease formation even in a relatively low concentration.

Threonine, which together with histidine affected the repression of B.cereus protease /Neumark and Citri, 1962/, also plays an important role in the regulation of protease formation in the used strain of B.megaterium.

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