PROTEASE REPRESSION IN BACILLUS MEGATERIUM KM

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The formation of enzymes in microbial cells is generally repressed by the products of their actions (Maas and Gorini, 1958, Jacob and Monod, 1961). Investigations were carried out to determine the influence of aminoacids in the medium on the synthesis of protease in cells of Bacillus megaterium KM and, further, to find out whether the enzyme accumulates in the process of its formation in some fraction of the cell. The sensitive method was applied in order to determine the proteolytic activity, using caseine, labelled with iodine ^{131}I as substrate (Chaloupka, Liebster, Janeček, 1958).

The strain of Bacillus megaterium, used in the experiment, was grown on a synthetic C/G substrate, containing mineral salts and glucose (McQuillen, 1955). A considerable amount of protease was formed under these conditions. The enzyme, secreted into the medium and released from the cells after their lysis with lysozyme (0.2 μg./ml.) showed optimal activity at approx. pH 7. Attempts to activate it by means of $1 \cdot 10^{-3}$ M cysteine and ascorbic acid did not meet with success. Lysozyme reduced protease activity by about 10%. An inhibitor present in the lysates of the cells and protoplasts reduces this activity by about 40%.

The localisation of the enzyme throughout the course of its synthesis and secretion into the medium was studied. The cells which had grown on C/G agar medium, containing 2% peptone, were washed and resuspended in C/G medium, containing $5 \cdot 10^{-3}$ M $CaCl_2$ and incubated at 32°C at 0.2 - 0.3/1 per min. aeration through a sintered glass. $CaCl_2$ was added in order to reduce protease inactivation (Vinter, 1956). Chloramphenicol (100 mg./ml.) was added to the collected samples (10 ml.) in order to prevent further enzyme synthesis. The samples were then centrifuged and the cells converted into protoplasts by lysozyme (Weibull, 1953) in 10 ml. of C medium, containing 0.25 M saccharose. The protoplasts were then centrifuged and disintegrated by osmotic shock in 10 ml. of C medium, containing $CaCl_2$. The proteolytic activity in the cultivation medium, in the supernatant after the centrifugation of the protoplasts, and in the lyzate of the protoplasts, was established. The resulting values were corrected to the inhibition caused by lysozyme and by the endogenous inhibitor, present in the protoplasts. No accumulation of the enzyme was observed either on the surface of the cytoplasmic membrane or inside the protoplasts(Figure I).

Enzyme formation ceased when 2% casaminoacids were added to the medium. The culture, used in the experiment, was grown in 4 flasks, containing 110 ml. C/G with $CaCl_2$. After one hour, a solution of casaminoacids to a concentration of 2% was added to 2 flasks while the same volume of C medium was added to the control flasks. To prevent foam formation and the denaturation of the enzyme in the medium, containing casaminoacids, 0.25 ml. of soya oil were added to each flask as an anti-foam agent and aeration was reduced to 0.05 l./min. Although these conditions resulted in slower growth and reduced

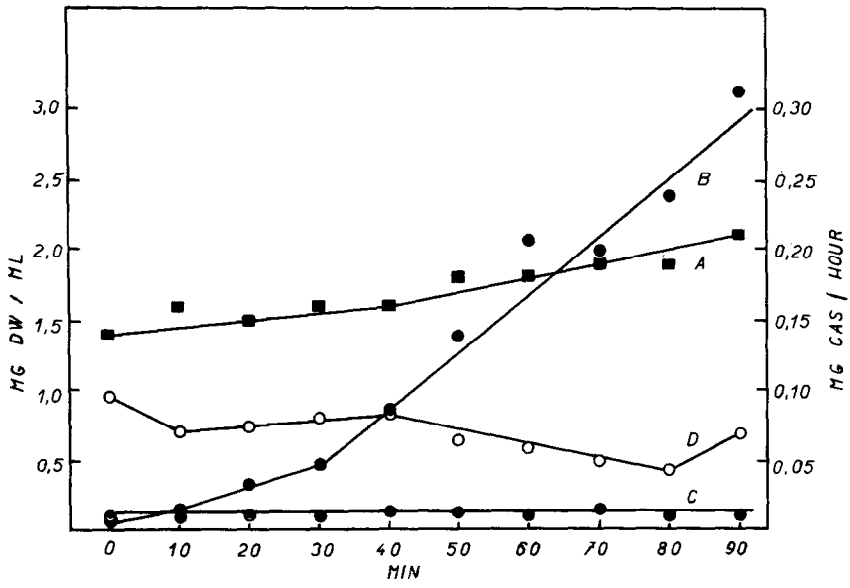

Fig.I. Protease localization during enzyme synthesis. The growth is expressed in mg. dry weight/ml. (A). Enzyme activity expressed in mg. of caseine/hour in ml. of medium (B), of the supernatant after protoplast centrifugation (C), of the cell lyzate (D).

enzyme formation, protease denaturation was reduced to less than 10%/hour.

Because the largest part of the synthesized enzyme was secreted into the medium, repression of protease formation was manifested chiefly by the low enzyme level of the medium.

The influence of amino acids on the level of the protease in cells was further established. The culture was grown either in C/G medium or in C/G medium with 2% casaminoacids. After the number of cells has increased twice and four times, samples were taken and proteolytic activities in disrupted protoplast were estimated (Table I).

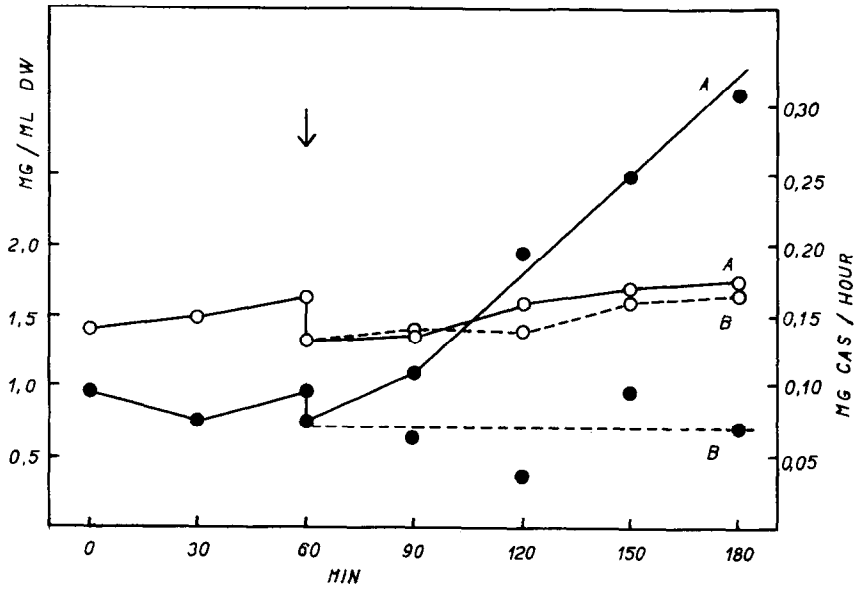

Fig.II. Repression of protease formation by 2% casaminoacids. A: growth in C/G medium. B: growth in the presence of 2% casaminoacids. The aminoacids were added at the moment indicated by arrow.
Open circles: dry weight in mg./ml.
Full circles: proteolytic activity in mg. caseine/hr. The values are equivalent to the sum total of the activity in 1 ml. of the medium and in the cells, which were grown in it. The results were corrected to the inhibiting activities of lysozyme, the endogenous factor and a 2% casaminoacids solution.

Table I

Experiment	Proteolytic activity in μg. cas/mg.protein/hr. at 37°C				
	Inoculum	1st doubling		2nd doubling	
	C/G + amino acids	C/G	C/G + amino acids	C/G	C/G + amino acids
1	8,4	5,4	5,2	5,1	6,4
2	11,1	6,7	4,9	7,4	5,1

The values are uncorrected.

Although the activities in cells were a little decreased in the culture grown in the presence of aminoacids, no real repression of intracellular enzyme synthesis was observed. It is assumed therefore, that the synthesis of extracellular and intracellular protease are regulated by different mechanisms.

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