AMINO ACID STIMULATION OF PROTEINASE SYNTHESIS IN A SPOROGENOUS BACILLUS MEGATERIUM KM.

Fayyaz Ud Din+ and J.Chaloupka
Department of General Microbiology Czechoslovak Academy of Science, Prague.

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Summary
The presented results indicate that the formation of an extracellular proteinase is stimulated by amino acids in one sporogenous strain of Bacillus megaterium KM. The stimulatory effect can be manifested in the absence of glucose only. The previously described regulatory mechanism suggested that amino acids or their metabolites are involved in the repression of proteinases in bacilli.

The formation of extracellular proteinase in different bacilli is inhibited in the presence of amino acids in the medium. This was found in different strains of Bacillus megaterium as well as in B. cereus (Chaloupka and Křečková 1962, Neumark and Citri 1962). However, amino acids or their metabolites do not appear to be the only candidates for the role of the effector as also glucose can suppress the enzyme formation (Laishley and Bernlohr 1966, Millet and Aubert 1969). The question therefore arises whether the synthesis of proteinases might be controlled by catabolite repression (Magasanik 1961). According to Levison and Aronson (1967) the regulation of proteinase might be effected by a common carbon catabolite the utilization of which as a material for the synthesis of amino acids is decreased in rich media. However, certain results indicate that both glucose and amino acids might be involved in the repression of the proteinase in bacilli, their different derivatives functioning in a cooperative manner (Chaloupka 1969).

+ UNESCO and University of 17.November graduate student

Material and Methods

The sporogenous Bacillus megaterium KM was a gift of Dr A.Aronson Purdue Univ. Laffayette, Ind. The concentrated suspension of spores in distilled water was kept at +5°C and used as the inoculum after thermal activation (15 min at 65°C).

The culture was cultivated in a complex peptone and glucose medium(Vinter 1960) with aeration at 35°C till the end of logarithmic growth. 5 ml of the grown culture (about 1 mg of dry weight/ml), were then diluted into 95 ml of a simple medium containing mineral salts and glucose. This medium contained the same mineral components as the complex one, however peptone was replaced with ammonium chloride (2g/l) and the concentration of glucose was increased to 2g/l. The cultivation continued in the shaking water bath at 35°C. Various amounts of amino acids (casamino acids Difco) were added at different time intervals.

The enzymic activity in the supernate was determined at pH 7,2 according to Charney and Tomarelli (1947), the unit of enzyme activity was defined in a previous paper (Chaloupka and Křečková 1966). Glucose in the medium was determined according to Somogyi (1945).

Results and Discusion

The organism does not produce substantial quantities of proteinase during growth in either complex or simple media. The enzyme is present in the supernatant of the complex medium only at the beginning of the stationary phase and its occurrence is indicated by the dissapearance of glucose. Almost no enzymic activity was found in the simple medium under similar conditions (Fig.1).

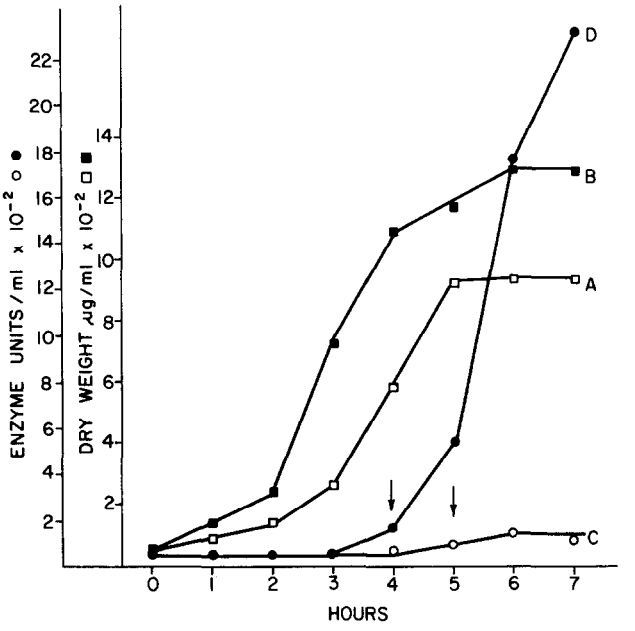

Figure 1. Formation of proteinase during growth.
The growing population was inoculated into mineral salts and glucose medium or the same medium enriched by 2 mg/ml of casamino acids (CAA). A: dry weight, C:proteolytic activity in the mineral medium with glucose, B: dry weight, D: proteolytic activity in the enriched medium. The arrows indicate the time when glucose was exhausted.

The development of proteolytic activity in the medium with amino acids was inhibited by chloramphenicol (100 µg/ml) added after 5 hours, indicating that the appearance of the activity was due to the synthesis of the enzyme. Equally, when amino acids (1 mg/ml) together with chloramphenicol (100 µg/ml) were added to the culture grown for 5 or 7 hours in the simple medium, no activity was found in the supernatant after further incubation (Table 1). This indicated again that the amino acids probably enhance the synthesis and not the activity of the proteinase.

The amino acids stimulate the synthesis of the proteinase to a greater extent when added at the end of the growth (Fig.2).

Table 1

Medium	A No additions	B CAA at 5 hrs	C CAA+CM at 5hrs	D CAA at 0 hrs CM at 5 hrs	E CAA+CM at 7 hrs
Enzyme U/ml 5 hrs	185	185	185	185	185
Enzyme U/ml 10 hrs	335	1250	185	185	185
Dry weight µg/ml 10 hrs	880	880	740	1000	830

The culture was grown in salts + glucose medium for five hours except for D, where casamino acids (1,0 mg/ml) were present from the beginning of the experiment. Additions were made after 5 hrs (B,C,D) and 7 hours (E) as indicated.
Abbreviations: CAA casamino acids 1 mg/ml, CM chloramphenicol 100 µg/ml.

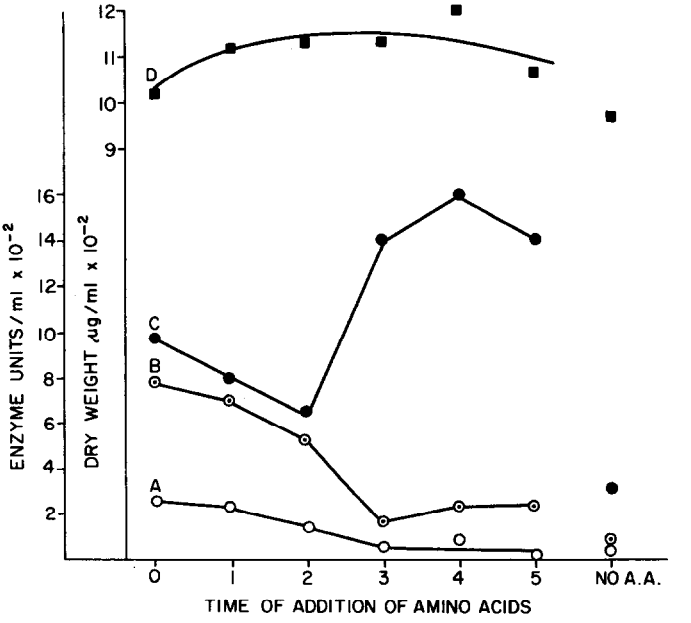

Figure 2. The influence of amino acids added at different time on the formation of proteinase.
0,5 mg/ml of casamino acids was added at time indicated to the population growing in salts plus glucose medium. Proteolytic activity was determined after 5 hours of growth(A), after 7 hours (B) and after 10 hours (C). Dry weight after 10 hours of growth is given (D).

However, their effect is manifested after a rather long lag period of more than two hours. Therefore it seems probable that they do not control the formation of the enzyme directly but via some metabolites, formation or accumulation of which begins after or must proceed for some time. The amino acids caused only a slight increase of dry weight as compared with the control. On the other hand they slowed down the development of spores together with increasing the synthesis of the proteinase when added after the third hour. The slight increase of proteolytic activity in the supernatant was observed also in the control culture incubated without amino acids for 10 hours. This indicates that also metabolites formed in the non growing population during the phase of the active turnover of protein

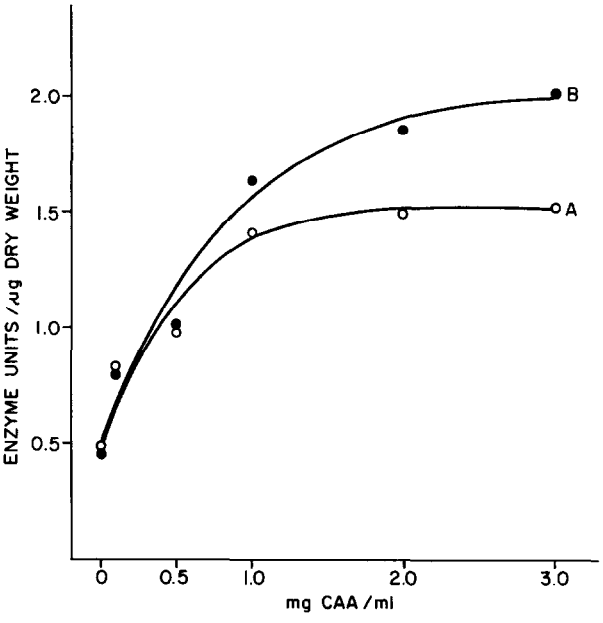

Figure 3. The influence of different amount of amino acids on the formation of proteinase.
The culture grew for 5 hours in the mineral salts plus glucose medium. After that time (the onset of the stationary phase) different amount of casamino acids was added. The proteolytic activity and dry weight were determined after 7(A) and 10 hours (B) respectively.

facilitate formation of the proteinase to a limited extent. The stimulatory effect on the synthesis of the enzyme can be shown after the addition of rather low amount of amino acids which have no measurable effect on the growth. The higher concentrations stimulate the formation of proteinase and the increase of cell mass as well. Figure 3 shows however that the increase of the enzyme is higher than that of the dry weight. The stimulation of proteinase synthesis by amino acids as described in this communication is at variance with previously suggested regulation of this enzyme in bacilli. However the presented results are similar with those of Jacoby and Gorini (1967) dealing with the contradictory effect of arginine on the control of arginine pathway in different strains of E.coli.

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