ACTION OF AMINO ACIDS ON THE VIABILITY OF THYMINE STARVED *Bacillus subtilis* CELLS.

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SUMMARY. A combination of two amino acids out of the following three: arginine, lysine, glutamic acid, effects a killing action on thymine starved cells thy⁻ of Bac. subtilis which generally remain viable if plated on a solid medium. This killing effect can be avoided by addition of any of the following amino acids: threonine, valine or leucine. If added together threonine and valine abolish mutually their rescuing action.

It was shown earlier (I) that Bacillus subtilis and Escherichia coli cells deficient in the synthesis of thymidilic acid (thy⁻) remain viable on a solid medium devoid of thymine if plated at moderate density: not more than 10^3 cells per square centimeter. If the seed is denser the cells die out. This finding provides a good possibility in studying the intrinsic features of the phenomenon of thymineless death.

In this paper we concentrate on the role of some amino acids on the viability of thymine starved cells.

EXPERIMENTAL. The strain I68 thy⁻ of Bac. subtilis was obtained by means of transformation of the strain I68 thy⁻ind⁻ of F. Rothman (2), the mutant I68 thy⁻arg⁻ was isolated from I68 thy⁻ after starvation for thymine on a solid medium (cf. ref. I). The strain of Escherichia coli used was B_3 thy⁻. Spizizen's growth medium I (3) supplemented with thymine (20 μg/ml) and (if necessary) with arginine (50 μg/ml) was used for cultivation. For testing different amino acids we used the same medium as for the cell growth but devoid of casein hydrolysate and other organic substances besides glucose. This salt-glucose

mineral medium we designate shortly as SGM.

Test for the action of amino acids on thymine starved cells.

Thoroughly washed Difco Bacto agar was used in preparing the SGM agar plates. A culture of thy$^-$ cells grown to 5 X 10^8 I/ml was washed from growth medium with SGM on a milipore filter, resuspended in SGM and plated on a series of Petri dishes at a density of 10 to 30 cells per square cm. The precise number of plated cells was established by titration on agar plates supplemented with thymine. The agar plates contained besides SGM the particular amino acid or combination of amino acids under investigation. A standard concentration of each amino acid was 10 µg/ml. After 20 hours of incubation at 32 C the agar discs were taken out of the Petri dishes and layered on top of agar plates with SGM supplemented with 20 µg/ml of thymine. The incubation was resumed till the appearence of colonies. The whole procedure was repeated with control plates which contained an equal seed of thy$^-$ cells on SGM agar plates but without amino acids. A comparison of colony counts on experimental and control plates gave a quantative measure of the influence of amino acids on the viability of thymine starved cells.

RESULTS and DISCUSSION. 18 amino acids were tested for their action on thymine starved thy$^-$ cells. Those were: histidine, methionine, tryptophan, phenylalanine, tyrosine, cysteine, hydroxyproline, lysine, arginine, aspartic acid, glutamic acid, serine, threonine, glycine, alanine, valine, leucine and isoleucine. Most of them have no specific action on thymine starved bacteria. The active amino acids can be divided into two groups. The first group includes arginine, lysine and glutamic acid. Any combenation of two of these amino acids gives a profound lethal effect on thy$^-$ cells of Bac. subtilis plated on agar medium devoid of thymine. Only arginine displays itself a moderate killing effect. Lysine and glutamic acid are

Table I.

EFFECT OF POISONOUS AND RESCUING AMINO ACIDS ON THE VIABILITY OF THE Bac. subtilis THY⁻ CELLS STARVING FOR THYMINE ON THE SOLID MEDIA.

Poisonous amino acids	V I A B I L T Y (percentage of cells forming colonies)	
	Poisonous amino acids only	Poisonous amino acids in the presence of leu, val or thr.
arg	I5 - 25	90 - I00
arg + glu arg + lys glu + lys	I - 3	90 - I00
glu-NH_2 + lys glu-NH_2 + arg	20 - 30	90 - I00

completely inert if taken alone (Table I). The efficiency of all three possible pairs of poisonous amino acids seems to be practically identical: only I - 3 % of plated cells remain viable after 24 hour exposure under standard conditions. The lethal effect is noticeable at much lower concentrations of amino acids (some tenth of a microgramm per ml.). It is important that in the presence of thymine in the agar medium any combination of poisonous amino acids cause no damage to thy⁻ cells. The poisonous action of some amino acids is strongly dependent on their structure: it was found that the substitution of glutamine for glutamic acid is much less effective. Aspartic acid or asparagine revealed no poisonous activity.

The second group of amino acids showing specific activity includes threonine, leucine and valine. Any of these amino acids eliminates the poisoning of thymine starved thy⁻ cells of Bac. subtilis by the amino acids of the first group. Threonine and valine display an antagonistic relationship: if mixed together they do not protect

thymine starved cells from poisoning by combinations of the amino acids of the group I. The chemical structure of amino acids seems quite specific for the rescue action: alanine and isoleucine which are closely related to valine and leucine are completely inert when tested for rescue action.

The rescuing activity of some amino acids could be possibly explained by interference with the active transport of the poisonous amino acids through the cell wall. To verify this hypothesis we performed following experiment. The strain of Bac. subtilis I68 thy$^-$ arg$^-$ was grown in SGM liquid medium supplied with 20 g/ml of thymine and with growth limiting concentrations of arginine. Leucine was added (20 µg/ml) to an aliquot of the initial culture and the kinetics of growth measured. We could expect in the last case a reduced rate of cell growth if leucine really prevents arginine transport. But nothing of the kind happened: the growth rate both in the presence and absence of leucine were the same (Fig. I). Therefore we conclude that the action of amino acids is the result

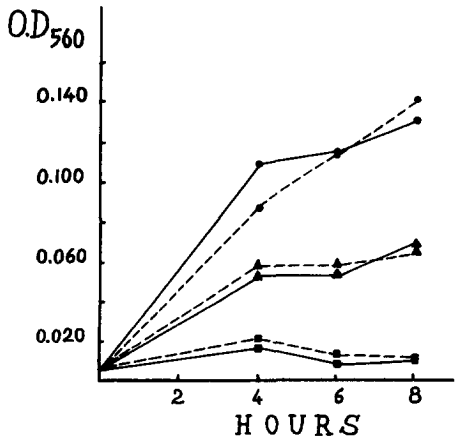

Fig. I.

GROWTH RATE OF THE STRAIN I68 thy$^-$ IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF ARGININE: —●—20,0 µg/ml; —▲—2,0 µg/ml; —■—0,2 µg/ml. Dotted curves - same amounts of arginine + 20,0 µg/ml of leucine.

of their interference with metabolic processes in thymine starving cells.

In a liquid medium it is impossible to study the effect of amino acids on thymine starving cells because they are subjected to thymineless death. The same is true if the culture is plated on SGM agar at a high density. The rescuing amino acids do not reveal any action in these conditions. We found that a special killing agent is excreted by the starving thy$^-$ cells and is the general cause of thymineless death. We shall discribe it in a later paper.

The observations presented here indicate that the mechanism of lethal action of poisonous amino acids is different if compared with common cases of thymineless death. Probably the thy$^-$ cells in conditions of thymine starvation undergo deep changes of amino acids metabolism which are revealed by the results described.

We tested also the action of all possible combinations of poisonous amino acids on cells of E. coli B_3 thy$^-$ starved for thymine on SGM agar plates but did not find any noticeable effect . This shows that the metabolic pathways in thymine starved cells are different in both species.

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