CATABOLITE REPRESSION OF "THREE SPORULATION ENZYMES" DURING GROWTH OF BACILLUS LICHENIFORMIS*

E.J. Laishley and R.W. Bernlohr**

Department of Microbiology
University of Minnesota
Minneapolis, Minnesota 55455

Received June 1, 1966

Bacillus licheniformis cells growing on glucose-salts medium synthesize arginine through glutamate via ornithine and other intermediates in the typical procaryotic manner (Vogel and Vogel 1963). After vegetative growth, sporulation is initiated concurrently with the appearance of a protease (Bernlohr 1964) that is thought to be necessary for providing endogenous amino acids for sporulation. In our laboratory, an arginase, ornithine-delta-transaminase and pyrroline-5-carboxylate dehydrogenase were shown to appear only during sporulation (Ramaley and Bernlohr 1966), and the degradation of arginine was postulated to procede to glutamic acid via ornithine and pyrroline-5-carboxylate. It has now been found that vegetative cells synthesize these enzymes when growing in the absence of glucose.

* This investigation was supported in part by grant AI-05096 from the National Institute of Allergy and Infectious Diseases.

** Recipient of Research Career Development Award, GM-K3-7709.

Induction of the arginase during presporulation. When B. licheniformis cells are growing on glucose medium + 10 mM arginine, the arginase can barely be detected (Fig. 1, 0—0). However, after the fifth hour, the glucose is exhausted from the medium as judged by the profile of the pH curve (Bernlohr and Novelli 1960), and the arginase is rapidly induced (●—●). This result suggests catabolite repression of the arginase induction by glucose. Similar results were obtained with cells growing on glycerol.

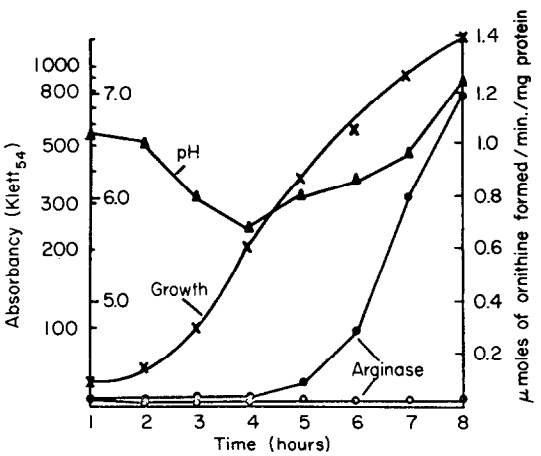

Fig. 1. B. licheniformis strain A-5 was grown in 50 ml lots in 60 mM glucose, 100 mM ammonium lactate and mineral salts medium (Bernlohr and Novelli 1963) supplemented with (●—●) and without (0—0) 10 mM L-arginine. Growth was at 37C with shaking in an Eberbach water bath shaker (120 one inch strokes per minute). Samples were removed at appropriate times and centrifuged at 29,000 x g at 0 C for 10 minutes. The supernatant solutions were discarded and cells suspended to 5 ml in fresh salt medium. The cells were broken at 2 C for 1 minute in the chamber of a Raytheon 50 W 9 kc/s sonic oscillator. The homogenates were dialyzed against two changes of 3 mM KH_2PO4 buffer pH 6.5 (5 hours each). Protein concentration was measured by the method of Lowry, et. al. (1951). Arginase was assayed by the appearance of ornithine from arginine by the method of Chinard as modified by Ratner (1962). The reaction mixture for the assay contains 100 µmoles tris buffer (pH 9.5) 10 µmoles L-arginine (pH 9.5) extract and water to 1.0 ml.

The Control of the Induction of the Arginine Breakdown Enzymes. The induction and catabolite repression of this induction can be demonstrated using cells growing on 50 mM sodium glutamate instead of glucose as the only carbon source. The generation time of the cells growing on sodium glutamate is about twice that on glucose and spores could not be detected in the growing cultures. Table 1 shows that arginine induces the arginase during exponential growth on sodium glutamate. If glucose is added to cells growing on sodium glutamate in the presence of arginine, this induction is repressed to the extent that by the time the

Table 1. Arginase Activity in Extracts of Cells Grown on Sodium Glutamate.

Time (hours)	Klett #54	Specific activity in μmoles/min/mg protein Control	+Arg.*	+Arg.*
2	18	0.40	3.04	2.20
4	24	0.85	11.5	12.4
6	48	0.20	21.5	23.8**
	**			
7	- 81	-	-	22.0
8	96 135	0.63	28.8	10.5
9	- 245	-	-	5.51

(*) Added to growth medium at 10 mM.
(**) 30 mM glucose added at the sixth hour.

Cells were grown as before in 200 ml lots in the minimal medium with 50 mM glutamate as sole carbon and nitrogen source, supplemented where noted with 10 mM L-arginine. Samples were removed at appropriate times, centrifuged at 10,400 x g at 0 C for 10 minutes. The supernatant solution was discarded and the cells suspended in a small volume of fresh salt medium. These cells were borken at 2 C for 2 minutes by sonic oscillation. The extracts were centrifuged at 22,000 x g for 10 minutes at 0 C, the sediments discarded, and the supernatant solutions dialyzed against two changes of 3 mM KH_2PO4 buffer pH 6.5 (2 hours each). The protein and arginase were analyzed as stated in Fig. 1. Spores were always germinated in the medium to be used experimentally, insuring that the inoculum (always 8%) contained cells that were physiologically similar to the test cultures.

cell number had doubled, the arginase activities in extracts had decreased to one half. Although there is an initial lag in the initiation of this repression, the enzyme is subsequently diluted as growth continues at a rate that is directly proportional to the generation time. This suggests that the repression of the arginase by glucose is extremely efficient. Cells grown on sodium glutamate without arginine have a significant level of arginase showing that added inducer is not necessary for the formation of the enzyme in growing cells. Similar results are obtained if sodium pyruvate or sodium acetate plus NH_4 ions are used instead of sodium glutamate as the carbon and nitrogen source.

In Table II, it is shown that both the L-ornithine-delta-transaminase and the pyrroline-5-carboxylate dehydrogenase activities are induced by arginine and repressed by glucose.

Table II. Ornithine-delta-transaminase and Pyrroline-5-Carboxylate Dehydrogenase Activities in Sodium Glutamate Grown Cells

		Transaminase		Dehydrogenase	
		Specific activity in μmoles/min/mg protein			
Time	Klett	Control		Control	
(hours)	#54	-Arg	+Arg*	-Arg	+Arg*
2	33		326	-	6.58
3	51	31.7	539	2.2	10.9
	- +G		- +G		- +G
4	77 93	-	561 403	-	17.2 13.1
5	108 222	25.0	795 222	2.5	24.9 10.1
6	140 450	-	888 137	-	- 8.15

(*) Added to growth media at 15 mM.
(+G) 30 mM glucose added at the third hour.
(-) No glucose added.

The procedure for growing these cells and for preparing cell free extracts were the same as reported in Table I. Assays for these enzymes were previously described by Ramaley and Bernlohr (1966). It was important to perform assays immediately after dialysis, as both activities are unstable.

Ramaley and Bernlohr (1966) demonstrated that these enzymes are absent or barely detectable in extracts prepared from B. licheniformis cells that were growing on glucose without arginine but were present in extracts of sporulating cells grown under the same conditions.

In contrast, it can be seen in Table II that the control (-Arg.) cultures exhibit a relatively high and constant level of both enzymes during growth in the absence of glucose. These activities can be increased at least 10 fold by the addition of arginine, and both the transaminase and dehydrogenase activities were subsequently diluted out upon the addition of glucose at 3 hrs. showing essentially complete repression of the snythesis of these enzymes during further growth. Reduced pyridine nucleotide dehydrogenase activity was constant under all growth conditions, allowing an adjustment of the pyrroline-5-carboxylate dehydrogenase activities in an unambigious manner.

Discussion. All of these data are consistent with the control of the entire degradative pathway of arginine to glutamic acid via the phenomenon of catabolite repression. When B. licheniformis is grown on glucose, the arginine catabolic enzymes are absent or barely detectable. When glucose is exhausted from the medium, the sporulation cycle is initiated with the concurrent appearance of the arginine catabolic enzymes; an arginase, ornithine-delta-transaminase and pyrroline-5-carboxylate dehydrogenase. In contrast, these enzymes are present in cells growing on a number of media in the absence of glucose. In other bacilli, a glucose mediated delay of sporulation has been observed by several other authors (see Schaeffer et. al. 1965). Catabolite repression of sporulation has been suggested by Schaeffer et. al. (1965) but very little work has been done on the control of the levels of enzymes during sporulation.

Since we have now shown that these arginine degrading enzymes, which normally appear during sporulation, are under catabolite repression control, it is important that supplementary criteria be used when relating the function of a post-exponential phase enzyme activity to sporulation. We would have normally considered these enzymes as being "sporulation specific enzymes." It is possible that they are, but it is also possible that many non-biosynthetic enzymes may appear in post-exponential phase cells as a result of the release of catabolic repression. The appearance of these activities appear during sporulation is not an a priori reason for assuming that they have a function in sporulation.

REFERENCES

Bernlohr, R.W., and Novelli, G.D., Arch. Biochem. Biophys., 87; 232 (1960).
Bernlohr, R.W., and Novelli, G.D., Arch. Biochem. Biophys., 103; 94 (1963).
Bernlohr, R.W., J. Biol. Chem., 239; 538 (1964).
Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem., 193; 265 (1951).
Ramaley, R.F., and Bernlohr, R.W., Arch. Biochem. Biophys., (in press).
Ratner, S., In Methods in Enzymology, ed. by S.P. Colowick and N.O. Kaplan, Vol. V, New York; Academic Press 1962 p. 843.
Schaeffer, P., Millet, J., and Aubert, J., Proc. Natl. Acad. Sci., 54; 704 (1965).
Vogel, R.H., and Vogel, H.J., Biochim. Biophys. Acta, 69; 174 (1963).