TEMPERATURE-DEPENDENT INDUCER REQUIREMENT FOR THE SYNTHESIS OF GLUTAMIC ACID DECARBOXYLASE BY ESCHERICHIA COLI*

Yeheskel S. Halpern

Department of Bacteriology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Received August 24, 1961

In his studies on amino acid decarboxylases of E.coli, Gale (1940a, 1940b) unequivocally established the inducible nature of these enzymes. A notable exception was the glutamic acid decarboxylase of strain 216 studied by this author, which seemed to be a constitutive enzyme. Addition of glutamate to the growth medium less than doubled the glutamic decarboxylase activity of the culture. On the other hand, while investigating the enzyme content of a number of E.coli mutants isolated by us (Halpern and Umbarger, 1961), we observed that in strains capable of producing glutamic decarboxylase, the enzyme was invariably of an-inducible nature. Comparison of the experimental procedure used by Gale (1940a) and that employed in our studies revealed a difference in the temperature at which the cultures were grown. In Gale's experiments the bacteria were grown at 27°, while our cultures were incubated at 37°. This report deals with the effect of temperature on the glutamate requirement for the synthesis of glutamic acid decarboxylase by E.coli.

---

* This work was supported in part by a grant from the Joint Research Fund, Hebrew University-Hadassah Medical School.

Methods. E.coli strain H/Gl$_1$ (Halpern and Unbarger, 1961) was used throughout this work. The medium employed was that of Davis and Mingioli (1950) from which citrate was omitted. Glucose, glutamate and other carbon compounds, where required, were added aseptically after autoclaving. Erlenmeyer flasks (250ml) containing 100ml of the desired medium adjusted to pH 5.8 (with bromcresol purple 0.001%, as indicator), were heavily inoculated (ca. 10^8 cells/ml) and incubated for 8-9 hours in a water bath with shaking (approx. 120 strokes/min.) at the desired temperature. The pH of the cultures was kept constant at 5.8 by addition of alkali or acid. The final trubidity usually was 0.80-0.90 measured at 550 mu in a Coleman Jr. Spectrophotometer. The bacteria were washed twice with saline, resuspended in distilled water and disrupted in a Mullard Ultrasonic Oscillator for 2 minutes. The debris were removed by centrifugation at 20.000xg. for 15 minutes, in the cold. The protein content of the extracts was determined by Mehl's biuret method (Mehl, 1945). The Glutamic decarboxylase activity of extracts was measured by the conventional manometric technique. The pool-size of free glutamic acid of cells grown at different temperature was determined by the method of Mandelstam (1958).

Results and discussion. In cultures grown at 37° the glutamic decarboxylase behaved as a typical inducible enzyme. Very little enzyme formed in the absence of glutamate, while upon addition of the latter, the decarboxylase activity of the cultures increased 7-17 fold. On the other hand, when grown at 30°, the bacteria formed large amounts of decarboxylase in the absence of glutamate and addition of glutamate to

the medium caused only a small increase in the amount of enzyme formed (Table 1).

TABLE 1

Effect of growth temperature on the formation of L-glutamic acid decarboxylase by E.coli H/Gl$_1$ in the presence and in the absence of L-glutamic acid.

Exp. No.	Q_{CO_2}			
	Cells grown at 30°		Cells grown at 37°	
	No glutamate added	0.5% L-glutamic acid added	No glutamate added	0.5% L-glutamic acid added
1	866	1131	40	773
2	1130	1408	160	1194
3	916	1345	98	883

The cultures were grown on 0.5% glucose; 0.5% L-glutamic acid was added where indicated. For other conditions see text.

* Q_{CO_2} = µl CO_2 released/mg protein/hr.

Since the amino acid decarboxylases of E.coli are thermolabile enzymes (Gale 1940a) it seemed possible that the effect of glutamate on the glutamic decarboxylase activity of cells grown at 37° was due to substrate-protection of the enzyme against heat-inactivation. The following experiments were carried out in order to check this assumption. Cell-suspensions with high decarboxylase activity were incubated for 4 hours at 30° and at 37° in a salt-glucose medium in the presence and in the absence of glutamate. Chloramphenicol (100 µg/ml) was added to the incubation mixtures to prevent cell-proliferation and protein synthesis. The decarboxylase activity of these suspensions was compared with that of an untreated control suspension. A similar experiment was carried out with incubation only at 37° in the absence of chloramphenicol. The results given in Tables 2 and 3 show that there was no appreciable

TABLE 2

Effect of temperature on glutamic decarboxylase activity of *E.coli* H/Gl_1 incubated with and without glutamate in the presence of chloramphenicol

	Q_{CO_2}			
Untreated suspension (control)	30°		37°	
	No glutamate added	In the presence of 0.5% L-glutamic acid	No glutamate added	In the presence of 0.5% L-glutamic acid
585	568	552	533	512

The bacteria were grown overnight at 37° on glucose and glutamate, 0.5% each. For other conditions see text.

TABLE 3

Effect of preincubation at 37° with and without glutamate in the absence of chloramphenicol on glutamic decarboxylase activity of *E.coli* H/Gl_1

Treatment	Initial turbidity (OD_o)	Final turbidity (OD_4hrs)	Factor of bacterial mass increase (OD_4hrs:OD_o)	Q_{CO_2}	Factor of decrease in decarboxylase activity (Q_{CO_2} of control:Q_{CO_2} of treated suspension)
Untreated suspension (control)	-	-	-	3654	-
Incubation at 37° in the absence of glutamate	.51	.63	1.23	2732	1.34
Incubation at 37° in the presence of 0.5% L-glutamic acid	.51	.73	1.43	2843	1.29

The bacteria were grown for 9 hrs at 30° on Na-succinate $.6H_2O$, 1% at pH 5.8. For other conditions see text.

inactivation of the enzyme at 37°. Consequently, one cannot explain the effect of glutamate at 37° by protection of the enzyme against inactivation. The enhancement of enzyme activity by glutamate must therefore be due to induction. The dependence of induction on temperature may be due to a lower level of internal inducer or to higher level of repressor in cultures grown at the higher temperature. The first possibility is unlikely, since as shown in Table 4,

TABLE 4

Effect of temperature on the pool-size of free glutamic acid in glucose-grown E.coli H/Gl$_1$

Incubation temperature °C	Growth rate (number of divisions per hr)	Free glutamic acid (in micrograms per 100mg dry weight of bacteria
30	0.59	158
37	0.67	199

The bacteria were grown on 0.5% glucose from an initial density of 0.08 and harvested when the cultures reached an OD of 0.51. Procedure used as described by Mandelstam (1958).

the pool of free glutamic acid is somewhat higher in cells grown at 37° than in those grown at 30°. The alternative assumption that less repressor is formed at 30° than at 37° is now under study.

REFERENCES

Davis, B.D. and Mingioli, E.A., J.Bacteriol., 60, 17 (1950).

Gale, E.F., (a) Biochem.J.(London), 34, 392 (1940).

Gale, E.F., (b) Biochem.J.(London), 34, 846 (1940).

Halpern, Y.S. and Umbarger, H.E., J.Gen.Microbiol., in press (1961).

Mandelstam, J., Biochem.J.(London), 69, 103 (1958).

Mehl, J.W., J.Biol.Chem., 157, 173 (1945).