THERMAL DEREPRESSION OF ALKALINE PHOSPHATASE SYNTHESIS

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Cases have recently been reported of temperature sensitive repression of enzym synthesis (Horiuchi et al., 1961; Halpern, 1961) and of vegetative phage developmen (Sussman and Jacob, 1962). The significance of such thermolabile repressor systems is that they provide a simple means of tampering experimentally with regulatory mechanisms whose response is normally exceedingly specific. In this communication we report the isolation of another such mutant, a strain of E. coli in which the repression of alkaline phosphatase synthesis by inorganic phosphate (Torriani, 1960) is strongly dependent on the temperature of incubation. Three other mutants have been obtained which show a weaker temperature effect.

Methods and Materials

The parental strain in these experiments was E. coli B3 (thymineless). Bacteria were cultivated in tris minimal medium (Gallant and Suskind, 1962) supplemented routinely with thymine at 10 micrograms per ml., potassium phosphate as indicated, and glucose or β-glycerol phosphate as indicated. Cell density was measured as optical density at 720 millimicrons (an O.D. of 1.0 at 720 millimicrons corresponds to 240 micrograms per ml. of TCA precipitable protein under these conditions). For enzyme assays, samples of the cultures were rapidly chilled, centrifuged, washed in cold 0.1 M Tris, pH 7.4, and resuspended in the same buffer. These suspensions were toluenized and assayed for alkaline phosphatase as described by Echols et al. (1961) except that the assay temperature was 25°. A unit of enzyme activity is defined as the amount of enzyme which produces a change of optical density at 410 millimicrons of 1.0 per minute at 25°. Specific activities are reported as enzyme activity divided by optical density at 720 millimicrons.

Results and Discussion

In setting out to isolate temperature sensitive repressor mutants, we took cognizance of the fact that base analogs often induce leaky mutational blocks. Since a mutant with a thermolabile repressor is by definition leaky, we screened base analog induced constitutive mutants for temperature sensitivity. Log phase E. coli B3 were grown for one generation in medium containing 50 micrograms per ml. 5-bromouracil and no thymine, then transferred to nutrient broth and grown for a further 1.5 generations. Alkaline phosphatase constitutives were selected for on tris minimal agar containing excess phosphate (2×10^{-3} M) and 1.5×10^{-2} M β-glycerol phosphate as sole carbon source (Torriani and Rothman, 1961). After 4 days at 37° the colonies which arose (frequency of 4.5×10^{-7}) were replicated to plates containing tris-glucose agar with excess phosphate and incubated at 25° and at 37°. Alkaline phosphatase production by the replicated mutants was measured crudely by spraying the plates with p-nitrophenylphosphate according to Echols et al. (1961). One colony, baptised PR_1, appeared to produce no phosphatase in plates incubated at 25° but substantial activity in plates incubated at 37°. Several other colonies showed a less marked activity difference in the two sets of plates. These colonies, as well as a few of the majority temperature independent ones, were picked and purified by two cycles of streaking and single colony isolation.

Table 1 presents the specific activities of alkaline phosphatase in cultures of several of these mutants grown in excess and limiting phosphate at 25° and 37°. PR_1 is clearly semiconstitutive at 37° and virtually completely repressible at 25°. PR_3 and PR_5 are fully constitutive at 37° but show a 50-60% reduction in specific activity in excess phosphate at 25°; PR_{11}, which is semiconstitutive at 37°, shows a similar 50% reduction at 25°. PR_2 and PR_8 are typical fully constitutive mutants showing no temperature effect.

Thermal derepression of PR_1 is illustrated in more detail in Fig. 1. The data are presented in the form of a differential plot of enzyme activity versus cell densi It is evident that, although the culture is derepressed by transfer from 25° to 37°, preheating in the absence of a carbon source produces no significant derepressic upon subsequent growth at 25°. It is therefore not surprising that rapid shift

Table 1.

Synthesis of alkaline phosphatase by B3 and constitutive mutants.

	Alkaline Phosphatase Specific Activity			
	25°		37°	
Strain	Limiting Phosphate	Excess Phosphate	Limiting Phosphate	Excess Phosphate
B3	0.46	<0.005	0.45	<0.005
PR_1	0.57	<0.005	0.65	0.24
PR_2	1.39	1.24	1.12	1.15
PR_3	1.17	0.27	1.04	0.78
PR_5	0.92	0.26	0.75	0.70
PR_8	1.36	1.39	1.03	1.36
PR_{11}	0.51	0.04	0.70	0.10

Cultures of the indicated strains were grown overnight at 25° and at 37° in medium containing limiting phosphate (5×10^{-5} M), and 0.2% glucose; and in medium containing excess phosphate (2×10^{-3} M) and limiting glucose (0.02%). Limiting growth was about the same for all strains under the same incubation conditions.

of the culture from 37° back to 25° results in the immediate reestablishment of repression. This is in marked contrast to the temperature sensitive repressor mutant described by Horiuchi et al. (1962), in which preheating results in derepressed synthesis of β-galactosidase for a considerable time after return to a low, normally repressing temperature.

The insert in Fig. 1 shows that the temperature of incubation has relatively little effect on the very low, repressed rate of enzyme synthesis in the parental strain. The curious initial rise in activity on transfer to a higher temperature has been repeatedly observed but is of unknown significance.

The enormous increase in alkaline phosphatase activity in cultures of PR_1 transferred from 25° to a higher temperature seems to be due to a bona fide increase in enzyme synthesis rather than enzyme activity. Heating the cells in

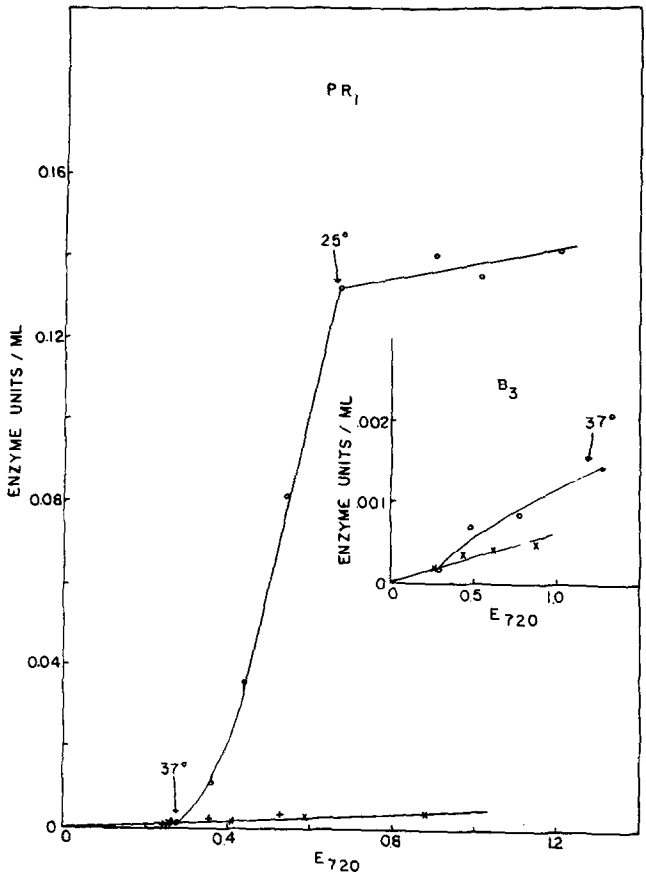

Figure 1. Thermal derepression of alkaline phosphatase synthesis. A culture of PR_1 was grown in excess phosphate (2×10^{-3} M) and limiting glucose (0.02%) until growth had stopped. At this point the culture was divided into three portions. The first portion was transferred to a 37° bath and 0.2% glucose added when the temperature of the culture reached 37° (open circles). A second portion was maintained at 25° and 0.2% glucose added (X). A third portion was heated for 60 minutes at 42° (during which no growth or alkaline phosphatase synthesis occurred) and then returned to 25° before adding 0.2% glucose (+). The culture transferred to 37° was rapidly chilled at the point indicated by the arrow (17 seconds immersion in an ice bath followed by transfer to a 25° bath); the temperature of the culture had reached 24.5° after one minute and the sample corresponding to the point immediately following the arrow was removed.

The insert shows data from an experiment performed in the same manner with a culture of B3. Culture maintained at 25° = X; culture transferred to 37° = open circles.

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buffer produces no increase in enzyme activity. Nor is any significant increase observed when the cells are transferred to a higher temperature in the presence of chloramphenicol. Extracts of cells grown at 25° do not inhibit the activity of extracts of cells grown at higher temperatures.

A limiting concentration of phosphate, 7×10^{-5} M, supports the growth of PR_1 and B3 at the same initial rate and for the same length of time before the break in the growth curve characteristic of phosphate depletion; phosphatase synthesis under these conditions is initially completely repressed in B3, partial repressed in PR_1, and increases to its fully derepressed rate of synthesis at the same time in both strains. It is therefore unlikely that the mutational lesion in PR_1 exerts its effect by controlling intracellular phosphate concentration.

Finally, we have observed that the differential rates of synthesis of two control enzymes (β-galactosidase and dihydroorotic acid dehydrogenase) show little response to the temperature of incubation in strain PR_1.

We conclude that the term thermal derepression is justified. The mutational lesion in PR_1 appears to specifically affect the rate of synthesis of alkaline phosphatase in such a way that its repression by inorganic phosphate is highly temperature sensitive.

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