Title

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Mary Eagleson and Vivian Moses

October 1966

Metabolite-Promoted Heat Lability of β-Galactosidase and Its Relation to Catabolite Repression

It has been reported by Gest and Mandelstam[1] that β-galactosidase from Escherichia coli is more heat-labile in the presence of fructose-1,6-diphosphate (FDP) than in its absence. They found the effect to be quite specific: of the other sugars and phosphorylated esters tested only glucose-6-phosphate (G6P) weakly, but significantly, increased the heat-lability of the enzyme. These results support the idea that β-galactosidase possesses a specific binding site for FDP. Gest and Mandelstam[1] suggested that FDP, which is a key intermediate in carbohydrate metabolism, might be the repressor metabolite postulated by Vogel[2] and Szilard[3]. Were this true, catabolite repression would be effected by the production of FDP, which would then combine with nascent β-galactosidase to prevent the enzyme from leaving the ribosomes on which it is being synthesized.

Prevost and Moses[4] observed that the intracellular concentrations of four compounds showed transient increases which paralleled kinetically the transient severe repression of β-galactosidase produced by adding glucose to cells growing on glycerol[5]. The four compounds were FDP, G6P, 6-phosphogluconic acid (6PG) and NADPH. We were interested in the possibility that the enzyme might possess "recognition" sites for all four of these compounds; 6PG and NADPH were not tested in the earlier work[1].

E. coli K-12 (strain Cavalli, obtained with thanks from Aleen Simmons) was used as the source of enzyme. The bacteria were grown

in glycerol-minimal medium, supplemented with thiamine, thymine and methionine. Enzyme synthesis was induced with 5×10^{-4} M isopropyl-β-D-thiogalactoside. One day old cultures were passed through the French press to disrupt the cells, and the resulting clear liquid used as a source of crude enzyme. Enzyme assay conditions are reported in the caption to Fig. 1.

Typical kinetic curves are plotted in Fig. 1. The accumulation of free o-nitrophenol in the last 8 min of the assay was used to measure the heat inactivation of the enzyme in the sample (Table 1). The results are expressed as percentages of the equivalent values for the control assay run simultaneously. The activity of the enzyme in the first 4 min of the assay gives, in most cases, some idea of the inhibitory (as distinct from heat-labilizing) or activating effects of each compound. The ratio of the activities in the last 8 min and the first 4 min of the assay is an indication of the extent of the heat denaturation which occurred. In some cases the activity in the first 4 min was so low that inhibition of the enzyme activity was suspected. Experiments run at 30°C, however, showed that ribose and NADPH had no effect at that temperature, and the same would probably hold for the other nicotinamide adenine dinucleotides and for the pentose phosphates. It seems therefore that these are very potent and rapidly-acting labilizers.

While our results in general agree with those of Gest and Mandelstam[1], we found that both fructose and 3-phosphoglyceric acid (3-PGA) were effective labilizers under our conditions. G6P as the barium salt labilized the enzyme; as the sodium salt it did not.

We suspect the labilizing effect in the former case was due to impurities in the preparation of the sugar phosphate.

Of the four compounds kinetically associated with catabolite repression in the experiments of Prevost and Moses[4], FDP and NADPH were effective labilizers, while G6P and 6PG were not. One might conclude from this that only the concentration changes shown by FDP and NADPH were casually related to catabolite repression, but the specificity of the heat-labilizing effect does not seem great enough to support such a conclusion. It must be noted that ribose, ribulose-1,5-diphosphate, ribose-5-phosphate, fructose, fructose-6-phosphate, NADH, NADP and 3-PGA, as well as the expected FDP and NADPH, caused the enzyme to become more heat-labile than usual. Ribulose-1,5-diphosphate is probably not an intermediary metabolite in E. coli. If, as Gest and Mandelstam[1] concluded, the heat-labilizing property of a small molecule indicates that the enzyme forms a specific complex with that molecule, then β-galactosidase must have recognition sites for several classes of compounds other than its substrate: pentose, pentose phosphates, nicotinamide adenine dinucleotides, fructose and fructose phosphates.

The suggestion of Vogel[2] and Szilard[3] that catabolite repression may be effected by an interaction between the catabolite corepressor and the nascent enzyme presupposes that catabolic regulation of enzyme synthesis is possible at the translational level, and is independent of the regulator (i) and operator (o) genes postulated by Jacob and Monod[6] as the regulatory site for enzyme derepression. Prevost and Moses[4] found that changes in the intracellular concentrations of their

four compounds correlated kinetically only with severe transient repression of β-galactosidase synthesis, and not with the moderate permanent repression which follows. Recent observations by Palmer and Moses[7,8] have shown that transient repression is effected via the i and o genes, while moderate permanent repression may be a non-specific consequence of changes in the biosynthetic balance of other proteins. Their results and the present findings thus lead to the conclusion that the interactions of β-galactosidase protein with various metabolic intermediates leading to changes in heat stability are probably not relevant to the phenomenon of catabolite repression.

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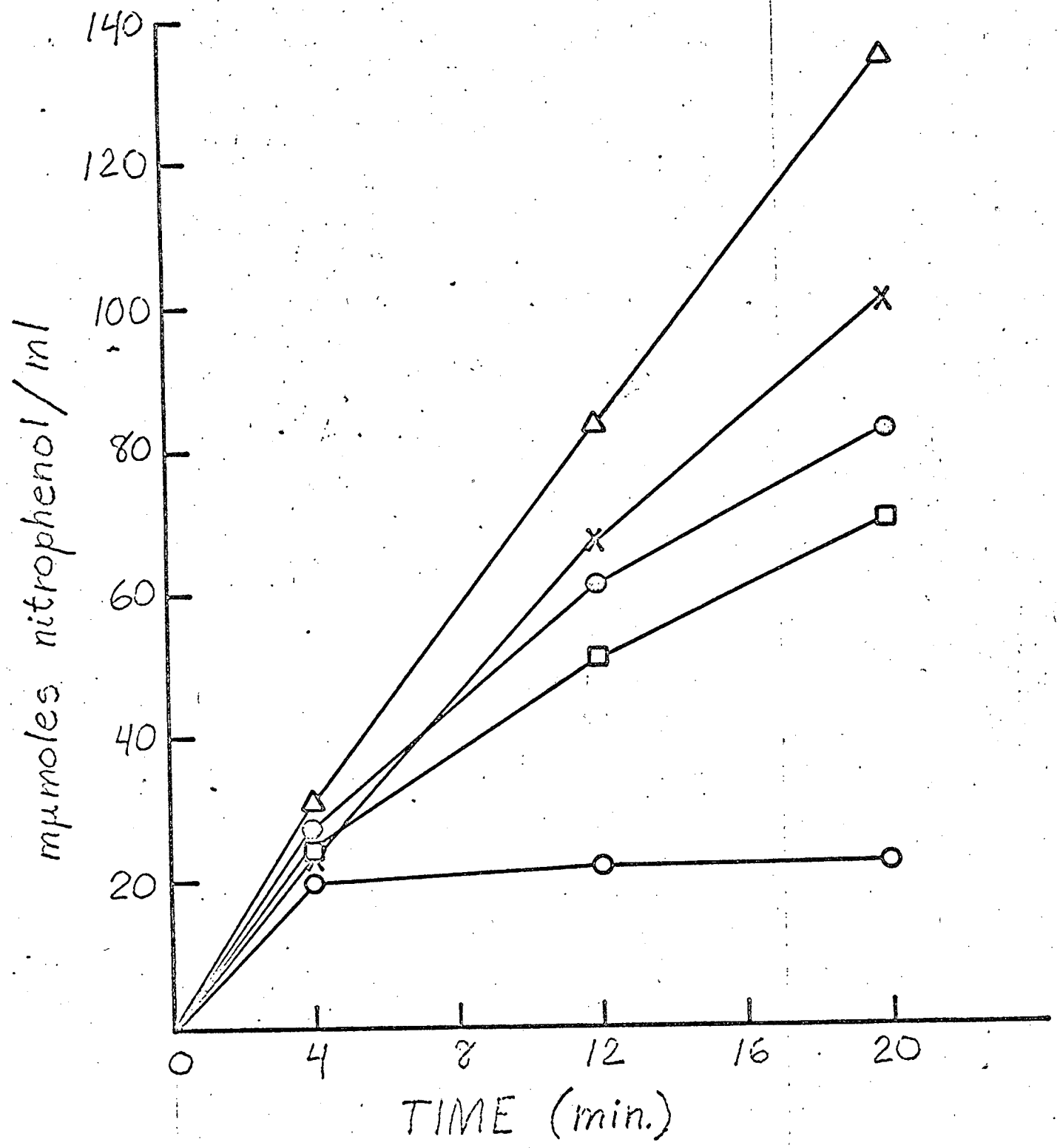
Table 1. HEAT LABILITY OF β-GALACTOSIDASE IN THE PRESENCE OF SEVERAL METABOLITES AT 48°

Compound	Rates of enzyme activity (as % of control) 0 - 4 min	12 - 20 min	$\frac{12-20}{0-4}$ (%)
FDP	119	32	27
Fructose-6-phosphate	27	1	3.7
Fructose	76	0.3	0.39
G6P	104	92	88
Glucose-1-phosphate	120	111	93
Glucose	116	98	84
6PG	77	60	78
Gluconic acid	107	105	98
NADPH	74	5.3	7.2
NADP	102	54	53
NADH	121	63	52
NAD	130	157	121
ATP	63	46	73
ADP	99	82	83
AMP	100	76	76
Ribose-5-phosphate	14	0.6	4.3
Ribulose-1,5-diphosphate	36	0.8	2.2
Ribose	49	8	16
3-Phosphoglyceric acid	96	34	35

Fig. 1. Effect of nicotine adenine dinucleotides on the thermostability of β-galactosidase at 48°. Enzyme was prepared as described in the text. Enzyme (0.01 ml) was added to 3 ml of 0.1 M sodium phosphate buffer (pH 7.0) containing o-nitrophenyl-β-D-galactoside (mM) and the test compound (2.5 mM). NADPH (⊙), NADH (●), NADP (⊡), NAD (△), or control (x). Aliquots (1 ml) were withdrawn at the times specified. The reaction was stopped with 0.2 ml of Na_2CO_3 (1.5 M) and the extinction read at 420 mμ.


140
120
100
80
60
40
20
mμmoles nitrophenol / ml
0
4
8
12
16
20
TIME (min.)