MECHANISM OF HYDROXYLAMINE-INDUCED INHIBITION OF β-GALACTOSIDASE SYNTHESIS IN ESCHERICHIA COLI

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Kepes and Beguin (1966) demonstrated an inhibition of induced β-galactosidase peptide synthesis by hydroxylamine (HA) without effect on messenger RNA (m-RNA) formation. The mechanism by which HA inhibits chain initiation is unclear, as is the question whether the effect is general or only specific for the β-galactosidase peptide. Experiments with E. coli strains K12 (λ) and 113-3 (a met, B12 auxotroph, Davis and Mingioli, 1950) indicated more severe inhibition of β-galactosidase synthesis by HA if glycerol, or similar metabolizable carbon sources are present in the medium.

This report demonstrates that in some strains, the HA effect involves catabolite repression in addition to interference with translation of the specific m-RNA. The use of HA as a specific inhibitor of chain initiation is thus limited in such strains.

Experimental: The methods of cell growth and of enzyme induction and assay have been described elsewhere (Basu et al., 1965). E. coli K12 (λ) was obtained from Dr. A. Weissbach of National Institute of Health, Bethesda, Maryland. Tryptophanase was induced and assayed according to McFall and Mandelstam (1963); one unit is that amount which forms 1 η mole of indole per minute in their assay.

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Results and Discussion: The effect of HA concentration on induced β-galactosidase synthesis by E. coli 113-3 is shown in Figure 1. In the presence of 10 mM glycerol the repression of enzyme synthesis is minimal in 0.01 mM HA but above 0.05 mM the repression is > 80%. With only citrate as carbon source, repression is 27% at 0.1 mM HA, whereas with increased glycerol concentration, the repression increases to a plateau of about 90% at ca. 10 mM glycerol.

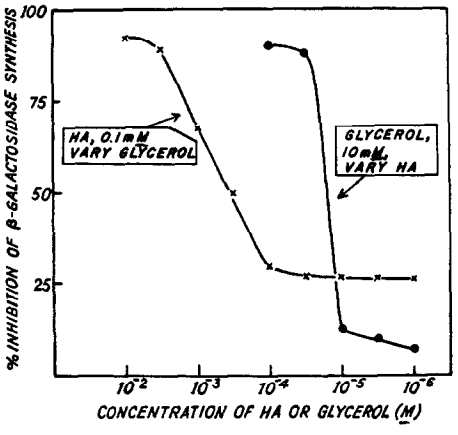

Fig. 1. Effect of concentration of HA and of glycerol on inhibition of β-galactosidase synthesis.

The lack of enzyme formation in E. coli 113-3 in the presence of HA does not result from cell killing. An experiment in the synthetic medium of Davis and Mingioli, with 10 mM glycerol and 5.0 mM TMG as inducer, samples taken at 10-minute intervals and plated, gave cell counts of 4.6 with and 4.5 X 10^8/ml without HA added at 0.1 mM, whereas in the latter, synthesis was inhibited by 90%.

A strong catabolite repression by glycerol, but not by casamino acids or citrate can be seen from Table 1. Only slight effect of HA was observed on the incorporation of ^{14}C-algal protein hydrolysate into cell protein irrespective of carbon source.

Table 1. Effect of carbon sources on β-galactosidase induction and on incorporation of ^{14}C-algal protein hydrolysate in E. coli 113-3.

Carbon source	β-galactosidase		^{14}c-incorporation	
	Hydroxylamine			
	-	+	-	+
	units/ml		c.p.m./mg protein	
Nil	21.9	16.1	5408	5274
Glycerol	129.2	9.7	5896	5560
Casamino acid	101.9	91.4	575	538
Citrate	40.3	30.3	——	——

In minimal medium + 100 μg/ml methionine, carbon sources were 10 mM excepting casamino acids, used at 0.5%.

Since catabolite repression is known to inhibit transcription only and not translation of preformed messenger (Nakada and Magasanik, 1964), the effect of HA was tested on both these phases of enzyme synthesis (Table 2). In measuring transcription, advantage was taken of the fact that PF quantitatively inhibits the synthesis of lac m-RNA in E. coli without having any effect on translation (Hurwitz and Rosano, 1965). It is clear that HA inhibits both the transcription and translation phases in presence of glycerol, but only translation in presence of casamino acids. The inability of casamino acids to enhance catabolite repression in some strains of E. coli has also been noted (McFall and Magasanik, 1962). The effect of HA on the translation is presumably due to inhibition of a chain initiation step (Kepes and Beguin, 1966).

The inhibitory effect of HA on other enzymes, like tryptophanase, an enzyme reported to be sensitive to catabolite repression (Freundlich and Lichstein, 1962), is shown in Table 3. Due to high basal level of this enzyme in strain 113-3, E. coli K12 (λ) was used. The action of HA is thus not specific for β-galactosidase, but acts also on hydrolytic enzymes like tryptophanase.

Table 2. Effect of HA on transcription and translation of lac operon in E. coli 113-3.

Expt. No.	Carbon source	Inhibition (%) Transcription	Translation
1	Glycerol	59	41
2		83	57
3		80	34
1	Casamino acids	-11	21
2		-2	31
3		-21	38

E. coli 113-3 cells were grown overnight in 20 ml synthetic medium, diluted 6-fold in same medium and grown an additional 3 generations. The cells were then harvested in the cold, washed and suspended approximately 5 X 10^8 cells/ml in fresh medium lacking glycerol or casamino acids. At 0-min induction of β-galactosidase was started by adding TMG and glycerol or casamino acids, with or without HA, PF, and CAP at 0 or 4 minutes. During the first 4 minutes of transcription detectable level of enzyme was not synthesized. The assay and the enzyme unit is as described earlier (Basu et al., 1965). Total period of incubation is 20 minutes. Although the results on three different days have been presented, the experiments were repeated at least seven times with more or less the same result. The effect of HA on transcription and translation phases has been calculated as follows: basal enzyme level (CAP at 0 minute) was subtracted from all systems. Then percent inhibition of translation:

$$= 100 \times \frac{\Delta \text{ Enzyme units with PF at 4 minute} - \Delta \text{ Enzyme units with HA and PF at 4 minute}}{\Delta \text{ Enzyme units with PF at 4 minute} - \Delta \text{ Enzyme units with CAP at 4 minute}}$$

In calculating HA effect on transcription, the inhibition of translation was taken into account. Thus percent inhibition of transcription by HA

$$= 100 - \frac{10000 \times \Delta \text{ Enzyme units with HA at 0 min, PF at 4 min}}{(100 - \% \text{ effect of HA on translation}) \times (\Delta \text{ Enzyme units with PF at 4 minute})}$$

Abbreviations: CAP, chloramphenicol; PF, proflavine; TMG, methyl-β-D-thiogalactoside

In synthetic media (Davis and Mingioli, 1950) HA will prolong the growth lag of both E. coli 113-3 and K12 (λ) from 2 hours to 3 to 4 hours. A shift down experiment was therefore designed to see if enhanced catabolite repression by HA might be due to its effect on growth (Paigen, 1963).

Table 3. Effect of carbon source on inhibition of tryptophanase synthesis by HA in E. coli K12 (λ)

Carbon source	Δ Tryptophanase units/ml (X100) HA -	HA +	Inhibition %
Nil	15.2	11.6	25
Glycerol	127.6	11.6	91
Succinate	107.6	1.9	98
Mannitol	138.9	8.6	96

All carbon sources at 10 mM, 1 enzyme unit = 1 n mole indole formed/min

Table 4. Effect of transfer from a rich to minimal medium on the HA inhibition of β-galactosidase synthesis in E. coli K12 (λ)

Growth period	Carbon source	Δ β-galactosidase units/ml HA −	HA +	Inhibition %
0 hr (Early lag)	None	1.6	0.1	94
	Glycerol	.6	0	100
3 hr (Late lag)	None	13.3	1.7	87
	Glycerol	22.7	1.6	93
6 hr (Mid log)	None	14.4	7.1	50
	Glycerol	9.4	3.0	69

Results in Table 4 indicate that cells harvested in lag phase from a shift down culture produced little enzyme, particularly in presence of HA, but those from mid log phase were less sensitive to HA inhibition. It is suggested that HA effect on catabolite repression may be due to an effect on growth of the organism.

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