ON THE REGULATION OF HISTIDINOL DEHYDROGENASE INDUCTION IN ARTHROBACTER HISTIDINOLOVORANS

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Summary

In order to investigate the control mechanism of histidinol dehydrogenase [HDH(I)] induction in Arthrobacter histidinolovorans, growth curves and induction experiments were carried out in presence of inhibitors of protein synthesis, namely chloramphenicol and actinomycin D. The evidence obtained from the gel electrophoresis patterns of the HDH activities in extracts of Arthrobacter cultures suggest that HDH(I) induction is regulated at the protein synthesizing complex level rather than at mRNA synthesis. A working model is proposed to explain the mode of control of HDH formation in this bacterium, which involves stable messenger formation and post-translation control by histidinol.

Arthrobacter histidinolovorans can produce two species of histidinol dehydrogenase (HDH) one, HDH(B) is produced constitutively, whereas the other, HDH(I), is synthesized only when histidinol is added to the growth medium. There is about 7-fold increase in total HDH activity in response to histidinol and this is not repressed by histidine, glucose + NH_4NO_3, glutamate or urocanic acid (1). Therefore, since two distinct HDH enzymes are involved, only one of which inducible, this HDH system in A. histidinolovorans appears to be different from that of the β-galactosidase, β-lactamase systems (2-4), or other known inductive systems. A large number of antibiotics are known to block the synthesis of proteins in bacterial cells. Among them, actinomycin D inhibits mRNA synthesis and chloramphenicol specifically inhibits the post-transcriptional stage of protein synthesis (5-7). In view of these findings

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experiments were carried out in order to throw some light on the mode of HDH(I) induction in A. histidinolovorans.

Materials & Methods

All chemicals used were of analytical grade. Pseudomonas mineral medium (PMM) consists of K_2HPO_4, 0.15%; KH_2PO_4, 0.05%; $MgSO_4 7H_2O$, 0.02% (8). Nutrient yeast broth (NYB): Nutrient broth (Difco), 0.8%; yeast extract (Difco) 0.5%. L-Histidinol dehydrogenase assay and gel electrophoresis were performed as described earlier (1). Protein was measured by the Folin phenol method (9) with the use of Bovine albumin fraction V (Sigma) as standard. Nutrient agar (NA): NYB + 1.0% lonagar (Difco).

Results

Growth Inhibition Studies

Growth inhibition curves for Arthrobacter were determined using inhibitors of protein synthesis. In these studies 20 ml PMM + 1% glucose + NH_4NO_3 (0.2%) in a series of side-arm flasks were shaken at 26°C after inoculating with 0.02 ml Arthrobacter culture in NYB grown overnight. At the midlog phase chloramphenicol and actinomycin D were added in various concentrations. Growth was monitored at intervals. Plots of E_{600} vs time indicate that maximal inhibition was obtained at a concentration of 25-50 µg/ml of chloramphenicol and at 10 µg/ml of actinomycin D. Concentrations lower than these levels do not show appreciable inhibition of growth. Results are illustrated in Fig. 1.

HDH Synthesis in Presence of Inhibitors of Protein Synthesis

50 ml PMM supplemented with 1% glucose, and 0.2% NH_4NO_3 in four side-arm flasks were shaken at 26°C after inoculating with a colony of Arthrobacter histidinolovarans grown overnight on an NA plate. Growth was monitored by measuring E_{600} values at intervals. When the cultures had reached midlog phase, inhibitors and histidinol were added as shown in Table 1. Cultures were harvested by centrifugation at 20,000 g for 15 min. Cells were suspended in 4 ml of tris-HCl buffer (pH 9.1, 0.05 M) containing 0.1% (v/v) 2-mercaptoethanol, and sonicated in an MSE 100 watt ultrasonic disintegrator for 2 min. each. The preparation was dialysed

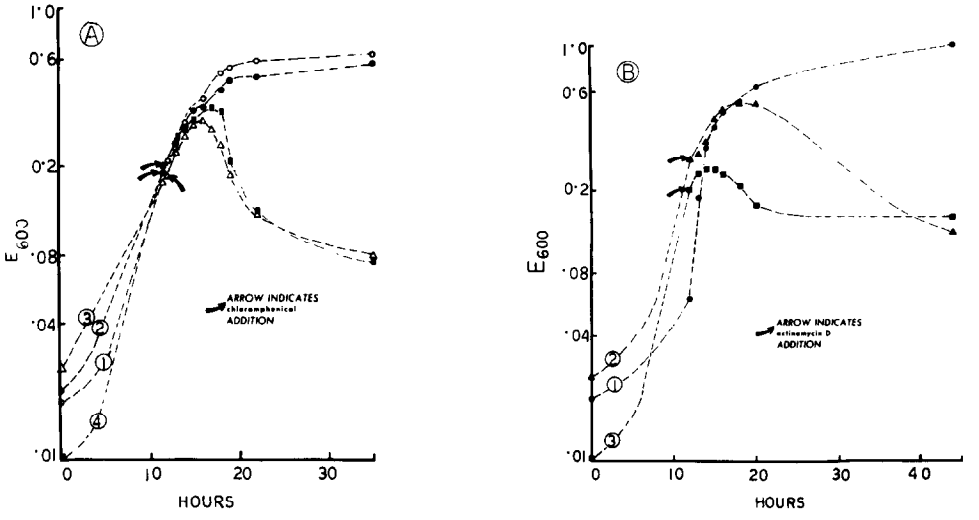

Figure 1. Inhibition of growth of Arthrobacter histidinolovorans. Plots of optical density (E_{600}) as a function of time in hours (semilog scale). A. 20 ml PMM supplemented with 1% glucose and 0.2% NH_4NO_3 in four side-arm flasks were shaken at 26°C. Additions of chloramphenicol are shown by arrow.

(1) No addition
(2) 5 µg/ml
(3) 25 µg/ml
(4) 50 µg/ml

B. Conditions are the same as A.

(1) No addition of actinomycin D
(2) 2 µg/ml
(3) 10 µg/ml

Table 1

HDH Formation in Arthrobacter Histidinolovorans in Presence of Inhibitors of Protein Synthesis

Flask No.	1*	2+	3+	4+
Additions	Histidinol (0.4 mg/ml)	Histidinol (0.4 mg/ml)	Histidinol (0.4 mg/ml) Chloram-phenicol (40 µg/ml)	Actinomycin D (10 µg/ml) histidinol (0.4 mg/ml)
E_{600} at harvest	0.185	0.620	0.335	0.350
HDH specific activity (mU/mg protein)	0.536	1.6	0.64	0.96
Total HDH activity (mU)	0.643	10.3	1.28	1.54

* Harvested immediately after histidinol addition.
\+ Harvested at the commencement of the stationary phase.

overnight against 100 ml phosphate buffer (pH 6.8, 0.01 M). Samples were tested for HDH activity and protein content (Table 1) and subjected to polyacrylamide gel electrophoresis.

The data summarized in Table 1 indicate that there is partial inhibition of enzyme formation both by actinomycin D and chloramphenicol. Although commencement of inhibition is not observed immediately after addition of inhibitors, specific activities and total activities in 3 and 4 are considerably lower than that of 2 (without inhibitor). Cells grown without inhibitor but with histidinol show two bands of HDH activity on gel electrophoresis (see gel scan of Fig. 2). In the case of chloramphenicol + histidinol grown cells, after its addition, E_{600} values go up from 0.195 to 0.335 but the gel electrophoresis pattern (gel scan 3 of Fig. 2) has only one significant band of activity corresponding to peak 1 in the gel scan and represents HDH(B). Actinomycin D was added when Arthrobacter cells had reached (E_{600}) 0.215; the cells continued to grow to (E_{600}) 0.275 and then stopped. After addition of histidinol, E_{600} values reached 0.350 and then growth stopped. The gel electrophoresis pattern of this preparation shows two bands of activity which correspond to HDH(B) and HDH(I) (gel scan 4 of Fig. 2) but the intensity of the faster moving band, (HDH(I), was not as great as that in gel scan 2. When cells were harvested immediately after addition of histidinol, the enzyme preparation showed two small peaks (gel scan 1 of Fig. 2) representing HDH(B) and (I).

The foregoing results with chloramphenicol suggest that chloramphenicol blocks the synthesis of HDH(I), even in the presence of histidinol. Although substrate-dependent activation of a protein precursor into an active enzyme has been demonstrated in bacteria (1)), in Arthrobacter such activation of macromolecular precursors (subunits or peptides) to give a fully functional (HDH(I) by histidinol does not seem to play a role. This assumption is based on the fact that if crude extracts of Arthrobacter culture are treated with histidinol prior to running the gels, they show only one band of enzyme

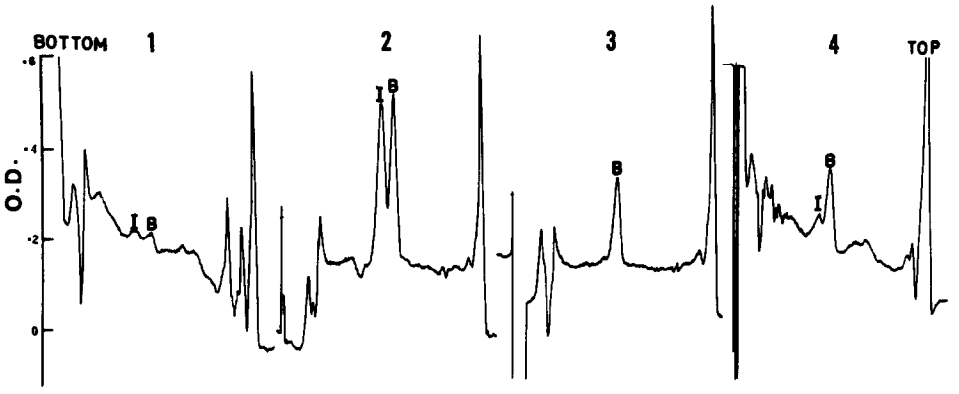

Figure 2. Spectrophotometric scanning of gels from polyacrylamide gel electrophoresis of HDH from Arthrobacter histidinolovorans. Gels, run with samples (50 µl/gel), were stained for HDH activity as described (1). They were scanned using a Gilford scanning assembly at 500 mµ (the scanning speed was 2 cm/min). The direction of the scanning was from top to bottom. Peaks represent HDH(B) or HDH(I). Cells grown in PMM + glucose + NH_4NO_3 plus addition, as indicated (Table 1).

(1) Harvested immediately after histidinol addition.
(2) Histidinol, harvested at stationary phase.
(3) Chloramphenicol + histidinol, harvested at stationary phase.
(4) Actinomycin D + histidinol, harvested at stationary phase.

activity, (HDH(B); furthermore, chloramphenicol would not be expected to block this type of (activation) synthesis.

Results with actinomycin D indicate that formation of HDH(I) is not dependent on messenger RNA synthesis and suggest the following possibilities: first, that a message may be produced with or without histidinol but that histidinol is essential for the translation of the mRNA which codes for the synthesis of HDH(I). Secondly, histidinol may reverse the action of actinomycin D. Thirdly, a stable message may be produced in the presence of histidinol. To test this last possibility, cells were grown in the presence of histidinol to induce message formation but its translation was blocked by chloramphenicol. Removal of chloramphenicol and histidinol would be expected to result in formation of HDH(I) if a stable message had been produced. Results indicated that after removal of chloramphenicol and histidinol, cells do grow further but do not produce HDH(I). This suggests that if HDH(I) synthesis involves stable message synthesis, it also needs the action of histidinol to initiate translation of the message into protein (inducer initiated translation).

A working model is proposed to explain the pattern of HDH synthesis in Arthrobacter histidinolovorans. According to this model, structural genes for HDH(B) and HDH(I) (may be adjacent to each other or many genes apart) are transcribed with or without histidinol to give stable messengers. Translation of mRNA for HDH(B) synthesis takes place without having any regulatory system, whereas translation of mRNA that codes for HDH(I) is independently regulated and is functional only in the presence of histidinol. Histidinol appears to act either as a stabilizer of the messenger or, possibly, by neutralizing the action of a repressor eventually resulting in HDH(I) synthesis. This appears to be a case of post-transcriptional control of enzyme synthesis which needs further exploration.

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