Induction and Repression of the Histidine-degrading Enzymes in Aerobacter aerogenes*

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L-Histidine is degraded by Aerobacter aerogenes in four steps to L-glutamate and formamide (1-3).

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\begin{align*}
L\text{-Histidine} & \rightarrow urocanate + NH_3 \\
\text{Urocanate} + H_2O & \rightarrow L\text{-4-imidazolone-5-propionate} \\
L\text{-4-Imidazolone-5-propionate} + H_2O & \rightarrow N\text{-formimino-L-glutamate} \\
N\text{-Formimino-L-glutamate} + H_2O & \rightarrow L\text{-glutamate} + \text{formamide}
\end{align*}
\]

It has previously been shown that the enzymes that catalyze the first two steps, L-histidine ammonia-lyase and urocanase, are induced by L-histidine and repressed by catabolites (4, 5). It will be shown in the present paper that the enzyme catalyzing the fourth step of the sequence, N-formimino-L-glutamate formiminohydroxylase, is also subject to induction by L-histidine and repression by catabolites. These observations raise the question whether the enzymes respond individually or as a unit to induction and repression. A possible approach to this problem suggested itself when it was found that the organism can grow with that of the first two enzymes.

**Experimental Procedure**

**Chemicals**—The preparation of the barium salt of N-formimino-L-glutamic acid is described in the preceding paper (3). The other chemicals were commercial preparations: l-aspartic acid, L-glutamic acid, L-histidine hydrochloride, and urocanic acid were obtained from Calbiochem; 2,6-dichlorophenol-indophenol, NADP, and diaphorase were products of Sigma.

**Bacteria**—The organisms used were A. aerogenes, strain 35 (7), and a related histidine-requiring mutant, strain 50 (8); they were maintained on nutrient agar slants.

**Preparation of Cell Extracts**—The cells were grown in the minimal medium previously described (8). The carbon and nitrogen sources were provided at a concentration of 0.2%. The cultures, 100 ml in l-liter Erlemeyer flasks, were inoculated with a liquid culture of cells grown in a similar medium to give an optical density, measured in a Klett-Summerson photoelectric colorimeter with a No. 42 filter, of approximately 20 Klett units, corresponding to approximately 10^9 cells per ml. The cultures were incubated with shaking at 37°C. The cells were collected by centrifugation at 6000 rpm in the cold from the exponential phase of growth when the optical density had reached 120 to 150 Klett units. The cells were washed twice with 20-ml portions of cold 0.01 M potassium phosphate buffer, pH 7.2. The pellets were stored overnight at 4°C; they were then suspended in 8 ml of the phosphate buffer and were disrupted by exposure to sonic vibration for 4 min in a Raytheon 10-Kc magnetostriective oscillator cooled to 1°C by an ethanol water bath. The debris was removed by a 15-min centrifugation at 40,000 rpm. The protein content of the extracts was found by the method of Lowry, et al. (9) to be 1 to 2 mg per ml. The extracts were immediately assayed for their enzyme activities.

**Enzyme Assays**—L-Histidine ammonia-lyase was determined by a slight modification of the method of Tabor and Mehler (10). The assay mixture contained in a total volume of 1.0 ml, 0.1 mmole of sodium pyrophosphate of pH 8.9, 2.5 μmoles of glutathione, and cell extract containing 0.02 to 0.1 mg of protein. The mixture was allowed to stand for 5 min at room temperature, then 10 μmoles of histidine were added and rate of increase in absorption at 277 μm, which is due to the appearance of urocanate, was measured at room temperature in a Zeiss spectrophotometer equipped with a Gilford recording attachment.

**Urocanase** was also determined by a slight modification of the method of Tabor and Mehler (10). The assay mixture contained,
in a total volume of 1.0 ml, 0.1 mmole of potassium phosphate buffer, pH 7.4, cell extract containing 0.03 to 0.2 mg of protein, and 0.075 μmole of urocanate. The rate of decrease of absorption at 277 μm was measured at room temperature in a Zeiss spectrophotometer with a Gilford recording attachment.

Formiminoglutamate hydrolase was determined as described in the preceding paper (9).

L-Glutamate: NAD(P) oxidoreductase (deaminating) was determined according to Sanwal and Lata (11); the assay mixture contained, in a total volume of 1 ml 80 μmole of Tris-HCl, pH 8.8, 0.2 μmole of 2,6-dichlorophenolindophenol, 2.5 μmole of NADP, 40 μg of diaphorase, and enzyme extract containing 10 to 50 μg of protein. The mixture was incubated at room temperature and the reduction of the dye was followed by measuring absorbance at 600 μm in a Zeiss spectrophotometer. When this endogenous reaction had come to an end, 2 μmole of L-glutamate were added and the rate of dye reduction was measured over the interval of 30 to 120 sec.

L-Aspartate ammonia-lyase was determined as described by Halpern and Umbarger (12).

L-Aspartate: 2-Oxoglutarate aminotransferase was determined by the spectrophotometric estimation of the oxaloacetate formed from aspartate in the presence of α-ketoglutarate and of pyridoxal phosphate as described by Cammarata and Cohen (13).

In all cases the enzyme activities were determined on duplicate samples of the extracts and were found to agree within 10%. Estimation of the specific enzymatic activities on separate extracts of cells grown in growth media of the same composition agreed within 25%. The enzyme levels reported in the tables are the average of two or more determinations carried out on such separately prepared cell extracts.

RESULTS

Growth of A. aerogenes on Urocanate.—The cell growing on histidine obtains neither energy nor biosynthetic intermediates from any of the reactions that bring about the conversion of L-histidine to L-glutamate and formamide. Moreover, since formamide is not metabolized (4), growth on L-histidine as far as energy or carbon compounds is concerned is equivalent to growth on L-glutamate. Therefore, compounds intermediate between histidine and glutamate should support the growth of the organism as well as histidine. However, this is not the case; the cells grown on urocanate only after a long lag at a very slow rate. When histidine-grown cells are transferred to a medium containing urocanate as the only source of carbon, growth begins after a short lag but proceeds at a rate (0.26 generation per hour) that is considerably slower than that obtained in a medium containing L-histidine as the only source of carbon (0.86 generation per hour). This observation suggests that urocanate cannot enter the cell rapidly enough to support growth at the same rate as histidine.

The extracts of the urocanate-grown cells contain not only urocanase and formiminoglutamate hydrolase, but also histidine ammonia-lyase, although this enzyme plays no role in the degradation of urocanate. The possibility had to be considered that the induction of the lyase is not directly brought about by urocanate, but rather that the enzyme acting in reverse converts urocanate and ammonia to histidine which in turn induces the enzyme. This possibility, which is a priori unlikely because of the apparent irreversibility of the lyase, was excluded by showing that urocanate alone or together with glycerol cannot support the growth of a histidine-requiring mutant of A. aerogenes, strain 50, even when this mutant, because of prior cultivation in a histidine-containing medium, is rich in histidine lyase.

The simultaneous induction of the lyase and the urocanase by urocanate would occur if both enzymatic activities were associated with the same protein moiety. This possibility cannot be brushed aside because, as will be discussed later, though the levels of these enzymes depend on the composition of the growth medium, their ratio is always constant (see Table I). However, it is possible to separate the lyase and urocanase present in a bacterial extract by chromatography. An experiment was carried out in which cells grown for 16 hours in a yeast extract-histidine medium were disrupted sonically and extracted; the extract was freed of the bulk of the nucleic acids by treatment with protamine sulfate (3). The supernatant was placed on a DEAE-cellulose column and eluted with the use of a gradient between 0.025 M and 0.25 M potassium phosphate, pH 7.2. Although a complete separation of the enzymes was not achieved by this method, the ratio of urocanase activity to lyase activity was increased from 0.1 in the crude extract to 1.1 in the fraction containing the highest amount of urocanase. The bulk of the urocanase activity was eluted considerably in advance of the lyase. The two enzyme activities appear therefore to be associated with different protein moieties.

Induction and Repression.—It could be shown that the three enzymes of histidine degradation, histidine lyase (I), urocanase (II), and formiminoglutamate hydrolase (IV), are inducible. Cells grown in a medium containing glucose and ammonia, or glycerol and ammonia, without histidine or urocanate contain a barely measurable level of enzyme I (0.04 μmole of histidine deaminated per min per mg of protein) and no measurable levels of enzyme II and enzyme IV. Cells grown in a medium containing histidine as the sole source of carbon, contain all three enzymes. It has previously been shown that the synthesis of histidine lyase is subject to repression by catabolites; the induced level of the enzyme is therefore greatly influenced by the composition of the growth medium. The levels of the three enzymes in cells grown in media of different composition were determined in order to discover whether the synthesis of enzyme II and of enzyme IV is regulated in a manner similar to that of enzyme I. In Fig. 1 the levels of urocanase (II) and of formiminohydrolase (IV) are related to the levels of the lyase (I) found in the cell extracts. The coordinate activities of urocanase and histidine lyase are readily apparent; on the other hand no obvious correlation seems to exist between histidine-lyase and formiminoglutamate hydrolase. It is nevertheless apparent that the induced level of the hydrolase is affected by the composition of the culture medium: it varies from 0.05 to 0.85 μmole of formiminoglutamate hydrolyzed per min per mg of protein.

The nature of the control of the synthesis of these enzymes becomes evident if we relate the enzyme levels to the composition of the growth medium (Table I). The levels of histidine lyase and urocanase are highest in a medium containing L-histidine as the only source of carbon; the substitution of urocanate for histidine lowers the levels of these enzymes by about 60%. The addition of glycerol to the histidine-containing medium, alone or together with NH₄⁺, depresses the levels of enzymes I and II to a greater extent than that of enzyme IV. The repressive effect of glycerol and NH₄⁺ on the first two enzymes is even
FIG. 1. Relation of level of urocanase and of formiminohydro- 
"lace to that of histidine lyase. The cells were grown in media 
containing histidine or urocanate alone, or together with glucose 
or glycerol, in the presence or absence of ammonium sulfate. 
The carbon and nitrogen sources were added to the medium at a 
concentration of 0.2%. Each condition is represented by one to 
four separate cultures. The cells were harvested during expo-

tional growth and disrupted. The levels of the three enzymes 
in each extract were measured and are given as units of enzyme 
per mg of protein.

TABLE I

<table>
<thead>
<tr>
<th>Composition of medium</th>
<th>Relative levels of enzymes</th>
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<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Histidine</td>
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</tr>
<tr>
<td>Histidine +</td>
<td>+</td>
</tr>
<tr>
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<tr>
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<tr>
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</tr>
<tr>
<td>Urocanate + Glucose</td>
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</tr>
</tbody>
</table>

* Omission of (NH₄)₂SO₄ does not affect these levels.
TABLE II
Response of aspartate ammonia-lyase and of glutamate dehydrogenase to changes in the growth medium

The levels of these enzymes were determined in extracts of cells harvested from the exponential phase in media of the indicated composition. The results shown are averages of the levels found in two to three separate cultures. The mean deviation is approximately 15%. The enzyme levels are given in units × 10^3 per mg of protein per min.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Composition of growth medium</th>
<th>Levels of enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>(NH₄)₂SO₄</td>
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<tr>
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<td>+</td>
</tr>
<tr>
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<tr>
<td>8</td>
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</tr>
</tbody>
</table>

* The organism, like most other enteric bacteria, does not grow readily in a medium containing glutamate as the only source of carbon.

The observation that the enzymes responsible for the degradation of histidine can be induced by urocanate raises the possibility that histidine is not an inducer in its own right, but acts as an inducer only by virtue of its conversion to urocanate. Cells grown in the absence of histidine do contain a low level of histidine ammonia-lyase and urocanase, and are thus capable of converting histidine supplied in the medium to urocanate which in turn could induce the entire set of enzymes required for the degradation of histidine.

DISCUSSION

The observation that the enzymes responsible for the degradation of histidine can be induced by urocanate raises the possibility that histidine is not an inducer in its own right, but acts as an inducer only by virtue of its conversion to urocanate. Cells grown in the absence of histidine do contain a low level of histidine ammonia-lyase and glutamate dehydrogenase. Glutamate does not induce the enzymes responsible for the conversion of histidine to glutamate, but rather plays a role in their repression (4). Histidine, on the other hand, appears to influence aspartate ammonia-lyase and glutamate dehydrogenase only by virtue of its conversion to glutamate. The third enzyme involved in the utilization of glutamate, aspartate aminotransferase, does not respond to these variations in the composition of the growth medium, its level in all cases was found to be 0.15 mmole of oxaloacetate produced per min per mg of protein.

The levels of these enzymes were determined in extracts of cells harvested from the exponential phase in media of the indicated composition. The results shown are averages of the levels found in two to three separate cultures. The mean deviation is approximately 15%. The enzyme levels are given in units × 10^3 per mg of protein per min.

The difference in the response of formiminohydrolase on the one hand and of histidine lyase and urocanase on the other indicates that the hydrolase does not belong to the same operon as the other two enzymes. The fact that histidine lyase and urocanase respond coordinately to changes in the level of the inducer as well as to changes in the level of the repressing catabolite suggests that the two control systems, induction and catabolite repression, both have as their target the same genetic unit, the operon.

SUMMARY

The response of three of the four enzymes responsible for the degradation of L-histidine in *Aerobacter aerogenes* to induction and catabolite repression was investigated. All three enzymes are induced by L-histidine and by urocanate and are repressed by glucose and by glycerol. The synthesis of the second enzyme of the pathway, urocanase, but not that of the fourth enzyme, formiminoglutamate hydrolase, is coordinated with that of the first enzyme, histidine ammonia-lyase.

REFERENCES
