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

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(Received for publication, March 30, 1965)

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

1. L-Histidine → urocanate + NH₃ (1)
2. Urocanate + H₂O → L-4-imidazolone-5-propionat (2)
3. L-4-imidazolone-5-propionate + H₂O → N-formimino-L-glutamate (3)
4. N-Formimino-L-glutamate + H₂O → L-glutamate + formamide (4)

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 formiminohydrolase, 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, NAD⁺, 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 1-liter Erlenmeyer 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 10⁷ 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; then 10 pmoles of histidine were added and rate of growth was measured at room temperature, then 10 pmoles of histidine were added and rate of increase in absorption at 277 nm, 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 (3).

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 grow 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 formimino-glutamate 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 hydrolized 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-
lase 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-
nential 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.

stronger when urocanate is the inducer; in this case, also, the
repressive effect on formiminoglutamate hydrolase is less marked.
The addition of glucose alone to the histidine-containing medium
causes a partial repression of all three enzymes; the addition of
uracil together with NH₄⁺ to a medium containing either
histidine or urocanate as the inducer brings about a strong repres-
sion of all three enzymes.

L-Aspartate Ammonia-lyase, L-Glutamate: NAD(P) Oxidoreduc-
tase, and L-Aspartate:2-Oxoglutarate Aminotransferase—The
products of the cleavage of L formiminoglutamate are L gluta-
mate and formamide (3); it is the further metabolism of gluta-
mate that enables the cell to grow on histidine or urocanate.
It seemed of interest, therefore, to determine the relation of
the control of the enzymes required for the degradation of glutamate
to that of the enzymes required for the conversion of histidine
to glutamate. It has been shown by Halpern and Umbarger
that in a strain of *Escherichia coli* growth on glucose and am-
monia produces cells rich in glutamate dehydrogenase and
relatively poor in aspartate ammonia-lyase, while growth on
uracil produces cells rich in the lyase and poor in the de-
hydrogenase (12). These findings support the concept that the
function of glutamate dehydrogenase is synthesis of glutamate
from the a-ketoglutarate produced from glucose and the NH₃ of
the growth medium. On the other hand, the function of aspar-
tate ammonia-lyase is degradation of glutamate. It serves this
function by converting to fumarate and NH₃ the aspartate
formed from glutamate by transamination; the fumarate is
converted to oxaloacetate, and in this manner permits the trans-
amination of glutamate to continue; NH₃ and ketoglutarate, the
other product of the transamination, serve the cell as sources of
nitrogen, carbon, and energy.

The results presented in Table II show that cells of *A. aerogenes*
grown on glucose and NH₃ are rich in glutamate dehydrogenase
and poor in aspartate ammonia-lyase, while histidine-grown
cells show the opposite pattern. The resemblance of the pattern
of the enzymes in histidine-grown *A. aerogenes* to glutamate-
grown *E. coli* is in good accord with the view that histidine is
metabolized exclusively by way of glutamate. Additional
experiments were carried out in an attempt to discover the role
of constituents of the growth medium in the repression and
induction of glutamate dehydrogenase and aspartate ammonia-
lyase (Table II). It appears that the addition of glutamate
(Experiment 2), or of histidine (Experiment 3), which is slowly
metabolized to glutamate in the presence of glucose and NH₃,
lowers the level of the dehydrogenase, and increases the level of

Table I

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<thead>
<tr>
<th>Composition of medium</th>
<th>Relative levels of enzymes</th>
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<tr>
<td></td>
<td>I</td>
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<tr>
<td>Histidine</td>
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</tr>
<tr>
<td>Histidine + Glycerol</td>
<td>29</td>
</tr>
<tr>
<td>Histidine + Glucose</td>
<td>13</td>
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<tr>
<td>Histidine + Glucose</td>
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<td>Urocanate + Glycerol</td>
<td>32</td>
</tr>
<tr>
<td>Urocanate + Glucose</td>
<td>6</td>
</tr>
<tr>
<td>Urocanate + Glucose</td>
<td>3</td>
</tr>
</tbody>
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* Omission of (NH₄)₂SO₄ does not affect these levels.
and urocanate may be due to the difference in the intracellular
the enzymes of histidine degradation to induction by histidine
pretation is equally plausible: the difference in the response of
entirely due to its conversion to urocanate, an alternative inter-

pmole of oxaloacetate produced per min per mg of protein.
grown in the absence of histidine do contain a low level of histi-
tion seems to suggest that the inducing effect of histidine is not
been induced with exogenous urocanate. Although this observa-

exogenous histidine, but considerably lower when the cells have

aspartate ammonia-lyase and glutamate dehydrogenase
repression (4). Histidine, on the other hand, appears to in-
formiminoglutamate hydrolase, is coordinated with that of the

of the pathway, urocanase, but not that of the fourth enzyme,

formiminoglutamate hydrolase is high, irrespective of whether the cells have been induced with exogenous histidine or urocanate; on the other hand, the levels of histidine lyase and of urocanase are high when the cells have been induced with exogenous histidine, but considerably lower when the cells have been induced with exogenous urocanate. Although this observation seems to suggest that the inducing effect of histidine is not entirely due to its conversion to urocanate, an alternative interpretation is equally plausible: the difference in the response of the enzymes of histidine degradation to induction by histidine and urocanate may be due to the difference in the intracellular urocanate levels. The slow growth of the cells on urocanate compared with the more rapid growth on histidine is a clear indication that exogenous urocanate enters the cell with difficulty; consequently in cells supplied with exogenous histidine the uro-
canate level is presumably higher than in cells supplied with exogenous urocanate. This high urocanate level may be neces-
ary for the full induction of histidine lyase and urocanase, while the low urocanate level in urocanate-grown cells may be sufficient for the full induction of the formiminoglutamate hydrolase.

The levels of histidine ammonia-lyase and of urocanase can be altered over a wide range by changes in the composition of the growth medium; however, the ratio of the levels of these two enzymes is always constant. Such coordinate behavior has been generally observed in the case of enzymes whose structures are determined by closely linked genes (14, 15). It has been postu-
lated that in these cases the segment of the deoxyribonucleic acid comprising the structural genes ("operator") responds as a unit to the product of a regulatory gene. This gene product ("repres-
sor"), presumably a protein, can combine with a metabolite or with a constituent of the growth medium ("effectuator"). In the case of inducible enzymes the inducer is the effectuator and serves to inactivate the active repressor; in the case of biosynthetic enzymes, the final product of the biosynthetic sequence is thought to be the effectuator and serves to activate the inactive repressor.

In both instances the active repressor combines with a portion of the DNA of the operon ("operator") and prevents the synthesis or release of a messenger ribonucleic acid replica of the operon. This messenger RNA in combination with the nonspecific parts of the protein-forming machinery (ribosomes and transfer RNA charged with amino acids) is responsible for the actual synthesis of the enzymes whose structures are determined by the operon (15).

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 catab-
olite 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**

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

Boris Magasanik, Patricia Lund, Frederick C. Neidhardt and David T. Schwartz