Imidazolepropionate, a Nonmetabolizable Inducer for the Histidine-degrading Enzymes in Aerobacter aerogenes*

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An important contribution to the study of inducible enzymes has been the observation that nonmetabolizable compounds similar in structure to the inducer may also cause induction (1-3). When the inducer is metabolized, the cell not only is provided with new enzymes but also with the products of the enzymatic action and ultimately with a new source of carbon and energy. With the use of compounds which can serve as gratuitous inducers, the specific phenomenon of enzyme induction may be dissociated from these other effects. The nonmetabolizable β-galactosides are the most familiar examples of such inducers. In Escherichia coli these compounds induce the enzyme β-galactosidase and a specific permease for galactoside transport (1,4-6). Because these galactosides accumulate unchanged in the cell they have been particularly valuable for the study of the specific permease (5-7).

In Aerobacter aerogenes the enzyme L-histidine ammonia-lyase is induced by histidine, its substrate, as well as by urocanate, the product of the irreversible reaction catalyzed by this enzyme (8). Although urocanate is a gratuitous inducer for the lyase it is further metabolized by the cell (9). The present work introduces the use of imidazole-4(5)-propionate as a nonmetabolizable inducer of the histidine-degrading enzymes. From the studies with this compound evidence was obtained for the existence of an inducible permease which appears to be specific for urocanate.

EXPERIMENTAL PROCEDURE

Organisms and Growth Conditions—A. aerogenes, strain 35, was used throughout these experiments. The medium has been described previously (10). Cells were grown at 37° on a rotary shaker bath, and growth was followed with a Klett-Summerson colorimeter (No. 42 filter) or a Zeiss spectrophotometer. Exponentially growing cultures were always used for measuring enzyme activities. The preparation of extracts has been described (11).

Enzyme Assays—The assays for histidine ammonia-lyase, urocanase, and formiminoglutamate hydrolyase have been described (8,12). In many experiments in which only the histidine lyase was assayed a modification of the method described by Hartwell and Magasanik (13) was utilized. Aliquots of the bacterial suspension to be assayed (about 0.04 mg, dry weight) were added to tubes containing 20 μg of CTB, 80 μmoles of sodium pyrophosphate, pH 10.2, and 10 μmoles of histidine in a final volume of 1.0 ml. The tubes were incubated at 30° for 1 hour when measuring the activity in fully induced cells, and for 5 hours when studying the initial kinetics of induction. The reaction was stopped by the addition of 0.5 ml of 10% perchloric acid. The cells were removed by centrifuging and the absorbance of the supernatant solution at 268 μm was determined. The linearity of the assay as a function of time and bacterial cell concentration is shown in Fig. 1. A unit of enzyme in this assay is defined as the amount which produces 1 μmole of urocanic acid per min.

Synthesis of ¹⁴C-labeled Imidazolepropionic Acid—A partially purified extract of A. aerogenes was prepared in the following manner. A culture of cells that had been grown with histidine as a carbon source was centrifuged, washed, resuspended in 0.05 M potassium phosphate buffer at pH 7.4, and sonically disrupted; the broken cell suspension was centrifuged; the supernatant fluid was treated with protamine sulfate (final concentration, 0.2%) and then heated to 80° for 10 min (14). This extract had no detectable urocanase activity. In later preparations a crude extract prepared from a mutant, M24, lacking urocanase was used. Uniformly labeled L-histidine, (10 to 100 μmoles, 2 to 20 μC per μmole) was dissolved in 5 to 10 μl of 0.04 M sodium pyrophosphate buffer and was incubated with a portion of the extract at room temperature. The amount of extract chosen was such that the conversion to urocanate, measured by the increase in absorbance at 277 μm, was complete in 1 to 3 hours. The reaction mixture was then placed in the side arm of a hydrogenating flask and evaporated to dryness in a vacuum. Platinum oxide (15) suspended in water was placed in the main compartment of the flask and was reduced with hydrogen gas. It was then mixed with the contents of the side arm, and the hydrogenation was continued with constant stirring at room temperature for several hours. The mixture was then filtered to remove the platinum, the filtrate was evaporated to dryness, and the residue was dissolved in a few milliliters of water. Denatured protein was removed by filtration. The absence of absorbance at 277 μm served as evidence that all urocanate had been reduced. The radioactive imidazolopropionate contained in the solution was purified on a Dowex 50-H⁺ column by the method described for the purification of urocanate (16). Descending chromatography on Whatman No. 1 filter paper in tert-butyl alcohol-formic acid.
have high levels of the enzyme histidine ammonia-lyase (8). In an attempt to find a nonmetabolizable inducer of this enzyme the bacteria were grown with succinate as a carbon source and the following compounds were tested: imidazole, imidazolepropionate, imidazoleacetate, L-2-thiolhistidine, L-imidazololactate, DL-α-methylhistidine, L-N-1-methylhistidine, L-N-3-methylhistidine, and DL-α-chlorimidazolepropionate. Only two compounds, α-methylhistidine and imidazolepropionate, induced the enzyme. An investigation of the induction by α-methylhistidine showed that cells grown in the presence of this compound have high levels of the histidine biosynthetic enzymes (11). Because of these elevated enzyme levels histidine is excreted into the medium and causes the induction of histidine ammonia-lyase. Imidazolepropionate did not affect histidine biosynthesis but appeared to act as a nonmetabolizable inducer of the lyase. The evidence that this compound is not metabolized is based on the fact that it did not serve as a source of carbon or nitrogen for this organism nor as a source of histidine for a histidine-requiring mutant. Furthermore, in experiments using the 14C-labeled compound the only radioactive compound isolated from the cells was imidazolepropionate (see below).

The intracellular level of histidine lyase as a function of the concentration of imidazolepropionate was determined by allowing cells to grow for many generations in the presence of imidazolepropionate. This level is referred to as the steady state value. As seen in Table I, the enzyme is induced by low concentrations of imidazolepropionate; the maximal level is achieved at a concentration of 0.1 mM. When the initial kinetics of induction was measured instead of the steady state value of the enzyme a different effect of inducer concentration was observed (Fig. 2A): imidazolepropionate at a concentration of 0.1 mM gave a much poorer induction than at the 10-fold higher concentration. Both the length of the lag period before enzyme appearance and the rate of enzyme synthesis were dependent on the inducer concentration. This finding suggests that a permease for imidazolepropionate is formed in response to this inducer and that it facilitates the induction of the enzyme.

It was also possible to determine the effect of histidine concentration on induction by following the early kinetics of induction.
followed in cells that had not been grown in the presence of imidazolepropionate; the steady state level of accumulation was reached within 5 min but only a small amount of imidazolepropionate was taken up. The permease activity in induced and uninduced cells was compared by measuring the total accumulation of radioactive imidazolepropionate as a function of its external concentration. As shown in Fig. 4A, induced cells take up approximately 5 times as much imidazolepropionate as uninduced cells. In some experiments an even greater difference was observed. Apparently imidazolepropionate not only induces histidine ammonia-lyase but also a permease responsible for transporting imidazolepropionate into the cells.

Permeases have been characterized by measuring an apparent dissociation constant ($K_d$) and a specific capacity, i.e., the maximal amount of a compound which the cell can accumulate (6, 19). These values were obtained for this permease by plotting the reciprocals of the internal and external concentrations of imidazolepropionate (Fig. 4B). The apparent dissociation constant for imidazolepropionate is 0.03 mM and the specific capacity of the fully induced cells at 37° is 5.7 μmoles of imidazolepropionate per g, dry weight.

**Fig. 2.** Induction of histidine ammonia-lyase. Cells were growing exponentially with 0.2% sodium succinate as carbon source. Inducer was added at zero time, and at the indicated times 0.2 ml was removed and pipetted into tubes containing CTB at 0°. The enzyme assay was carried out for 5 hours at 30°. A, induction by imidazolepropionate. ○—○, 1.0 mM; ▲—▲, 0.5 mM; △—△, 0.1 mM; ▼—▼, 0.04 mM; ■—■, 0.01 mM; □—□, 0. B, induction by histidine. ○—○, 0.1 mM; ▲—▲, 0.04 mM; △—△, 0.01 mM; ▼—▼, 0.004 mM; ■—■, 0.01 mM; □—□, 0.

**Fig. 3.** The uptake of $^{14}$C-imidazolepropionate as a function of time. Cells were grown overnight in 0.2% sodium succinate and 1.0 mM imidazolepropionate. They were then diluted in the same medium and allowed to grow for several generations. They were centrifuged, washed with 0.85% sodium chloride, and resuspended in minimal medium to a concentration of 0.40 mg per ml, dry weight. Cells were incubated at 37° in the presence of 100 μg of chloramphenicol per ml and imidazolepropionate (specific activity, 106 cpm per μmole). Aliquots (1 ml) were removed at the indicated times and filtered. ○—○, 0.01 mM imidazolepropionate; ▲—▲, 0.01 mM imidazolepropionate plus 10 mM sodium azide.
The uptake of \( {\text{\textsuperscript{14}}C}\text{-imidazolepropionate} \) as a function of external concentration. A, induced cells were prepared as described in Fig. 3. A parallel uninduced culture was treated in the same manner. Cells were incubated in the presence of the radioactive compound, and 100 \( \mu \)g per ml of chloramphenicol for 5 min at 37° before being filtered. \( \bullet \)--\( \bullet \), induced cells; \( O \)--\( O \), uninduced cells. B, reciprocal plot of the uptake of imidazolepropionate as a function of external concentration for induced cell.

Several aromatic amino acids and other imidazole compounds were also tested for their ability to displace imidazolepropionate from the cells (Table II). None of the amino acids tested had any effect on the level of imidazolepropionate accumulated by the cells. The only imidazole compound, other than urocanate, that was able to displace imidazolepropionate was \( \alpha \)-chlorimidazolepropionate. This compound did not induce the histidine lyase, but it inhibited the induction of the enzyme by imidazolepropionate.

The permease appears to be specific for imidazolepropionate and for urocanate. A direct comparison of the uptake of \( {\text{\textsuperscript{14}}C}\text{-urocanate} \) in induced and uninduced cells was not made because the urocanate is degraded in the induced cells. The uptake of radioactive urocanate was followed in a urocanase-negative mutant of \( A. \) aerogenes which has a constitutive permease (22). The uptake of imidazolepropionate and urocanate were similar in this organism. The specific capacity for urocanate in cells grown on succinate is 5 \( \mu \)moles per g of dry weight and the apparent dissociation constant for urocanate is 0.002 \( mM \).

Inhibition of Urocanase—All of the imidazole compounds that had been tested as inducers were also tested as inhibitors of histidine ammonia-lyase and of urocanase. In these experiments histidine lyase was assayed by first adding the analogue at a concentration of 2.5 \( mM \), and then adding the substrate, histidine, at a concentration of 1.0 \( mM \). Urocanase was assayed in the same manner, except that the concentrations of analogue and substrate were 0.5 \( mM \) and 0.05 \( mM \), respectively. Of these compounds only imidazolepropionate was found to have any effect; it is a competitive inhibitor of urocanase. The activity of urocanase as a function of substrate concentration in the presence or absence of imidazolepropionate was measured and the data were plotted according to Lineweaver and Burk (21) (Fig. 6). The \( K_i \) for imidazolepropionate is 1.9 \( \times 10^{-4} \) \( mM \), and \( K_m \) for urocanate is 4.8 \( \times 10^{-6} \) \( mM \).

Induction and Repression of Permease and Histidine-degrading Enzymes—In the previous paper the effect of varying the carbon source on the levels of the histidine-degrading enzymes was determined with either histidine or urocanate as inducer (8).

### Table II

Specificity of imidazolepropionate permease

<table>
<thead>
<tr>
<th>Addition</th>
<th>Time after addition of ( {\text{\textsuperscript{14}}C}\text{-compound} )</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1 min</td>
</tr>
<tr>
<td>None . . . . . . . . . . .</td>
<td></td>
</tr>
<tr>
<td>L-Tryptophan . . . . . . .</td>
<td>100</td>
</tr>
<tr>
<td>DL-( \alpha )-Methylhistidine . . . . . .</td>
<td>102</td>
</tr>
<tr>
<td>L-Phenylalanine . . . . . .</td>
<td>107</td>
</tr>
<tr>
<td>( \alpha )-Chlorimidazolepropionate . . . . . .</td>
<td>48</td>
</tr>
<tr>
<td>L-Histidine . . . . . . . . . . . . . . . .</td>
<td>108</td>
</tr>
<tr>
<td>Urocanate . . . . . . . . . . . . . . . .</td>
<td>46</td>
</tr>
<tr>
<td>( {\text{\textsuperscript{14}}C}\text{-imidazolepropionate} ) . . . . . .</td>
<td>44</td>
</tr>
</tbody>
</table>
Some of these experiments were repeated in order to include a study of the permease and to compare the effect of imidazolepropionate as inducer with that of histidine and urocanate. Cells that have been grown with glycerol as carbon source have a very low level of permease which is increased by growth in the presence of any of the three inducers (Table III). The level of permease is actually higher in cells grown with succinate in the absence of inducer than in cells grown with glycerol plus inducer. The addition of either histidine or urocanate to cells grown with succinate further increases the permease level. Cells grown with glucose as carbon source in the presence or absence of inducer had essentially no permease activity. The large changes in specific capacities shown in Table III were not accompanied by noticeable changes in the dissociation constant of the permease.

The levels of the histidine-degrading enzymes under various growth conditions and with different inducers were also compared (Table IV). The specific activities of the enzymes from cells grown with histidine as carbon source were taken from the data presented in the previous paper (8). These values have been set at 100. The first point to note is that imidazolepropionate not only induces histidine lyase but also urocanase and formimino-glutamate hydrolase. Secondly, it can be seen that histidine lyase, urocanase, and the permease, but not formimino-glutamate hydrolase, are greatly repressed when cells are grown in the presence of glycerol. With succinate as carbon source and imidazolepropionate as inducer, the permease and formimino-glutamate hydrolase levels are comparable to those obtained with histidine-grown cells but the levels of histidine lyase and urocanase are low.

### DISCUSSION

The results presented in the preceding section have shown clearly that imidazolepropionate induces A. aerogenes to form the enzymes required for histidine degradation, as well as a permease required for concentrating imidazolepropionate and urocanate inside the cell. The same enzymes and the permease...
are also induced by histidine and by urocanate (8). The inducible pernese does not appear to be necessary for the uptake of histidine; rather this amino acid can be transported into the cell by another, constitutive, pernese. Thus the formation of the imidazolepropionate or urocanate pernese is a gratuitous event when histidine is the inducer, but not when imidazolepropionate is the inducer. The formation of the enzymes, on the other hand, is gratuitous when imidazolepropionate is the inducer since this compound is not metabolized.

Imidazolepropionate acts as an analogue of urocanate and not of histidine; it is transported into the cell by the same pernese that handles urocanate and it is a competitive inhibitor of the enzyme urocanase. It is most probable that it induces the histidine-degrading enzymes by mimicking the action of the natural inducer, urocanate. However, because of the inhibitory effect of this analogue on urocanase another explanation must be considered. A small portion of the endogenously produced histidine may be normally metabolized through the action of the histidine-degrading enzymes. The inhibition of urocanase by imidazolepropionate could then bring about an intracellular accumulation of urocanate, which in turn would serve as the inducer for the histidine-degrading enzymes. This interpretation is not in agreement with the observation, reported in the succeeding paper (22), that imidazolepropionate is able to induce urocanase in a mutant which, because of the low level of histidine lyase, is unable to produce urocanate from histidine. Therefore imidazolepropionate must be considered as an inducer in its own right of the histidine-degrading enzymes. The fact that this non-metabolizable compound can induce not only urocanase, the enzyme whose substrate it mimics, but also histidine lyase and formiminoglutamate hydrolase is a clear indication that the induction of these enzymes is not sequential (23); rather all of the enzymes must respond to a single inducer.

In the preceding paper the response of the histidine-degrading enzymes to induction by histidine and by urocanate and to repression by catabolites derived from different carbon sources has been described. The formation of urocanase, but not of formiminoglutamate hydrolase, was shown to be coordinated with that of histidine lyase. A similar pattern of coordination has now been observed when the enzymes are induced with imidazolepropionate. The urocanate pernese responds to the same inducers but does not appear to be formed coordinately with either the histidine lyase or the formiminoglutamate hydrolase. This pattern of coordination suggests that the structural genes for these enzymes and the pernese are grouped into three different units all of which responds to the same inducer.

Histidine can be concentrated by A. aerogenes when the cells are grown under conditions in which the histidine-degrading enzymes are not formed (unpublished experiments) (see also Reference 22).

**summary**

The nonmetabolizable compound, imidazolepropionate, has been shown to induce the enzymes that degrade histidine in *Aerobacter aerogenes* by acting as an analogue of urocanate. From studies with imidazolepropionate evidence is presented for the induction of a specific urocanate pernese which is induced under the same conditions as the histidine-degrading enzymes but does not appear to be coordinated with either the histidine lyase or the formiminoglutamate hydrolase.

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**REFERENCES**

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