Biochem. J. (1973) **132**, 423–433
Printed in Great Britain

The Control of the Enzymes Degrading Histidine and Related Imidazolyl Derivatives in *Pseudomonas testosteroni*

By J. G. COOTE* and H. HASSALL
Department of Biochemistry, University of Leeds, 9 Hyde Terrace, Leeds LS2 9LS, U.K.

(*Received* 31 *August* 1972)

1. The induction of the enzymes for the degradation of L-histidine, imidazolylpropionate and imidazolyl-L-lactate in *Pseudomonas testosteroni* was investigated. 2. The activities of histidine ammonia-lyase, histidine–2-oxoglutarate aminotransferase and urocanase are consistent with these enzymes being subject to co-ordinate control under most growth conditions. However, a further regulatory mechanism may be superimposed for histidase alone under conditions where degradation of histidine must take place for growth to occur. 3. Experiments with a urocanase$^-$ mutant show that urocanate is an inducer for the enzymes given above and also for *N*-formiminoglutamate hydrolyase and *N*-formylglutamate hydrolase. 4. *N*-Formiminoglutamate hydrolase and *N*-formylglutamate hydrolase are also induced by their substrates, and it is suggested that these two enzymes may be different gene products from those expressed in the presence of urocanate. 5. Induction of the enzyme system for the oxidation of imidazolylpropionate is dependent on exposure of cells to this compound.

The pathway of L-histidine degradation has been elucidated for *Pseudomonas fluorescens* (Tabor & Mehler, 1954), *Salmonella typhimurium* (Magasanik, 1963), *Klebsiella aerogenes* (Lund & Magasanik, 1965), *Pseudomonas aeruginosa* (Lessie & Neidhardt, 1967), *Bacillus subtilis* (Chasin & Magasanik, 1968) and *Pseudomonas testosteroni* (Coote & Hassall, 1973). *P. fluorescens*, *P. aeruginosa* and *P. testosteroni* degrade L-histidine in five steps to L-glutamate and NH_3, as follows:

$$\text{L-Histidine} \rightarrow \text{urocanate} + NH_3 \quad (1)$$

$$\text{Urocanate} + H_2O \rightarrow \text{3-(imidazol-4-on-5-yl)propionate} \quad (2)$$

$$\text{3-(Imidazol-4-on-5-yl)propionate} + H_2O \rightarrow \textit{N}\text{-formimino-L-glutamate} \quad (3)$$

$$\textit{N}\text{-Formimino-L-glutamate} + H_2O \rightarrow \textit{N}\text{-formyl-L-glutamate} + NH_3 \quad (4)$$

$$\textit{N}\text{-Formyl-L-glutamate} + H_2O \rightarrow \text{L-glutamate} + HCO_2H \quad (5)$$

The enzymes catalysing the above reactions are (1) histidase (L-histidine ammonia-lyase, EC 4.3.1.3), (2) urocanase, (3) imidazolonylpropionate amidohydrolase (EC 3.5.2.7), (4) *N*-formiminoglutamate hydrolase, and (5) *N*-formylglutamate hydrolase. The other organisms use a similar pathway, but possess one less enzyme. They convert *N*-formiminoglutamate directly into L-glutamate and formamide.

Studies on the regulation of the synthesis of three of the enzymes of the pathway (histidase, urocanase and formiminoglutamate hydrolase) have been reported for *K. aerogenes* (Schlesinger *et al.*, 1965), *P. aeruginosa* (Lessie & Neidhardt, 1967) and *B. subtilis* (Chasin & Magasanik, 1968). In these organisms all three enzymes are induced by growth on L-histidine or urocanate and are subject to repression by catabolites. In all cases, the synthesis of histidase appears to be co-ordinately controlled with that of urocanase, but not with that of formiminoglutamate hydrolase. From experiments with mutants lacking either histidase or urocanase, evidence has been presented implicating urocanate as the physiological inducer for these enzymes in *K. aerogenes* and *P. aeruginosa*, and L-histidine as the natural inducer in *B. subtilis*.

Schlesinger & Magasanik (1965), using *K. aerogenes*, and Lessie & Neidhardt (1967), using *P. aeruginosa*, have shown that imidazol-5-ylpropionate can act as a gratuitous inducer for the enzymes degrading L-histidine in these organisms. Its action is attributed to its chemical similarity to urocanate so that it mimics the action of the natural inducer. Imidazolylpropionate does not serve as a gratuitous

* Present address: Division of Microbiology, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, U.K.

inducer for the enzymes in *B. subtilis* (Chasin & Magasanik, 1968), which supports the proposition that L-histidine is the natural inducer in this organism.

In contrast to any of the organisms discussed above, *P. testosteroni* is able to utilize imidazolylpropionate or imidazolyl-L-lactate (as well as L-histidine or urocanate) as sole source of carbon for growth (Hassall & Rabie, 1966; Hassall, 1966; Coote & Hassall, 1973). Imidazolyl-L-lactate is initially oxidized to imidazolylpyruvate, which is then converted by L-histidine–2-oxoglutarate aminotransferase into L-histidine. This is subsequently degraded to L-glutamate. Imidazolylpropionate is converted into urocanate without the participation of L-histidine as an intermediate. *P. testosteroni*, therefore, has a greater versatility than other organisms by virtue of its ability to utilize the additional imidazolyl derivatives as sources of carbons for growth. We report here a study of the regulation of the L-histidine-degrading enzymes in this organism. This includes an examination of the last enzyme of the pseudomonad pathway, formylglutamate hydrolase, the regulation of which has not been previously studied, and also the aminotransferase that is involved in the metabolism of imidazolyl-L-lactate.

Materials and Methods

Organisms

The organism used throughout these studies was originally isolated from soil by its ability to use imidazolylpropionate as sole source of carbon for growth and was identified as *P. testosteroni* (N.C.I.B. 10808). A mutant strain lacking urocanase (A26), and a 'leaky' histidase mutant (11/13), possessing less than 10% of the inducible histidase of the wild-type organism, were isolated after treatment of the parent strain with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as described previously (Coote & Hassall, 1973). A mutant (13/18) that had lost the ability to grow on imidazolylpropionate was also used.

Methods

Cultivation of organisms. Cells were grown at 30°C in a medium containing (g/litre): KH_2PO_4, 2; $(NH_4)_2SO_4$, 1; $MgSO_4,7H_2O$, 0.2; carbon source, 2; the pH was adjusted to 7.0 with 5M-NaOH. When histidine was used as the sole source of nitrogen $(NH_4)_2SO_4$ was omitted from the above medium. Normally, 10ml of medium was inoculated from a stock slant and incubated with shaking at 30°C. The resulting culture was used to inoculate a further 90ml of medium. This in turn was incubated with shaking at 30°C and the cells were harvested while in the exponential phase of growth.

Preparation of cell extracts. Harvested cells were washed and resuspended in 0.1M-KH_2PO_4 buffer (adjusted to pH7.0 with 5M-NaOH) and were broken by ultrasonic disintegration as described in the preceding paper (Coote & Hassall, 1973). The supernatant fraction obtained after centrifugation at 38000*g* for 30min was used. Protein concentration was determined by the method of Lowry *et al.* (1951) with a reference standard of bovine serum albumin.

Enzyme assays. Histidase activity was assayed by a modification of the method of Tabor & Mehler (1955) with the pyrophosphate buffer replaced by 2-amino-2-methylpropane-1,3-diol buffer. The assay followed the formation of urocanate by measuring the rate of increase in E_{277}. This and other spectrophotometric assays were carried out at 30°C in a Gilford 2000 multiple-sample absorbance recorder fitted with a Unicam SP.500 monochromator. The reaction mixture contained 10μmol of L-histidine, 10μmol of GSH, 1ml of aminomethylpropanediol buffer (0.1M-aminomethylpropanediol adjusted to pH9.2 with 2M-HCl), 0.1ml of enzyme preparation and water to 3ml. The reaction was started by the addition of L-histidine after preincubation of the enzyme in the presence of GSH for 15min.

Urocanase activity was assayed by measuring the rate of decrease in E_{277} of a reaction mixture containing urocanate (Tabor & Mehler, 1955). The reaction mixture contained 0.2μmol of urocanate, 1ml of phosphate buffer (0.1M-KH_2PO_4 adjusted to pH7.0 with 5M-NaOH), 0.1ml of enzyme preparation and water to 3ml. Histidase and urocanase assays on samples of the same preparation were usually run simultaneously. Control reaction mixtures were used that lacked substrate. L-Histidine–2-oxoglutarate aminotransferase activity was assayed by following the formation of the enol–borate complex of imidazolylpyruvate at 293nm on incubation of the enzyme with L-histidine and 2-oxoglutarate in borate–arsenate buffer (Coote & Hassall, 1973).

Formiminoglutamate hydrolase activity was assayed by measuring the rate of disappearance of formiminoglutamate from a reaction mixture, by using the method of Rabinowitz & Pricer (1956) to measure formiminoglutamate. The reaction mixture contained 1.5μmol of formiminoglutamate, 1ml of Tris buffer (0.1M-Tris adjusted to pH8.0 with 2M-HCl), 1ml of enzyme preparation and water to 5ml. Samples (1ml) were withdrawn at time-intervals up to 1h for the measurement of formiminoglutamate.

Formylglutamate hydrolase activity was assayed by a method based on that of Ohmura & Hayaishi (1957), which measures the appearance of the ninhydrin-positive product of the reaction, glutamate. The reaction mixture contained 0.75μmol of formylglutamate, 0.025μmol of $FeSO_4,7H_2O$, 1ml of 0.1M-Tris–HCl buffer (pH7.0), 0.5ml of enzyme preparation and water to 2.5ml. Samples (0.5ml) were removed into 1ml of 2% (w/v) trichloroacetic acid at intervals up to 30min. The precipitated protein was removed by centrifugation and 0.5ml of

supernatant solution was neutralized with 1M-KOH. The volume of this was made up to 1ml with water and glutamate assayed by the procedure of Rosen (1957).

Enzyme units. One unit of enzyme activity is defined as the amount of enzyme catalysing the reaction of 1 μmol of substrate/min at 30°C. Specific activities are expressed as munits/mg of protein. For the calculation of enzyme activities the following values for the molar extinction coefficients were used: enol–borate complex of imidazolylpyruvate, ϵ_{293} 12000 (Spolter & Baldridge, 1963); urocanate, ϵ_{277} 18800 (Tabor & Mehler, 1955).

Uptake of ^{14}C-labelled compounds by whole cells. The uptake of ^{14}C-labelled compounds by whole cells was measured as described by Coote & Hassall (1973). Cells were removed by filtration of the medium through Oxoid cellulose acetate discs and radioactivity was assayed in a Nuclear–Chicago gas-flow counter.

Chemicals

Forminimoglutamate was obtained as the barium salt (BDH Chemicals Ltd., Poole, Dorset, U.K.) and was converted into the sodium salt by the addition of an equivalent amount of Na_2SO_4; $BaSO_4$ was removed by centrifugation. Imidazolylpropionate, urocanate and formylglutamate were prepared as described previously (Coote & Hassall, 1973). Tris, L-histidine and 2-oxoglutarate were obtained from Sigma (London) Chemical Co., London S.W.6, U.K. 2-Amino-2-methylpropane-1,3-diol was obtained from Kodak, London W.C.2, U.K. All other chemicals were obtained from BDH Chemicals Ltd.

The buffers used were as described in the preceding paper (Coote & Hassall, 1973).

Results

Growth response of the organism to various substrates

The rates of growth of the organism in liquid culture with various compounds as sources of carbon, and $(NH_4)_2SO_4$ as the source of nitrogen, were determined spectrophotometrically. The mean generation times were: L-glutamate, 1.6h; succinate, 1.8h; L-histidine+succinate (with L-histidine as the only nitrogen source), 1.9h; *N*-formyl-L-glutamate, 1.9h; *N*-formimino-L-glutamate, 2.0h; urocanate, 2.0h; imidazolylpropionate, 2.3h; L-histidine, 4.7h; imidazolyl-L-lactate, 5.0h.

Induction of the enzymes of the histidine catabolic pathway

The enzymes considered for study were four of the five enzymes involved in the conversion of L-histidine into L-glutamate, and L-histidine–2-oxoglutarate aminotransferase, which catalyses the formation of histidine from imidazolylpyruvate during the metabolism of imidazolyl-L-lactate (Coote & Hassall, 1973). The activity of imidazolonylpropionate amidohydrolase was not measured, since the substrate for the enzyme is unstable and this leads to difficulty in developing a sensitive assay system. The enzymes converting imidazolyl-L-lactate into imidazolylpyruvate

Table 1. *Effect of carbon source during growth on the subsequent specific activities of enzymes of the pathway of L-histidine catabolism in P. testosteroni*

In the L-histidine+succinate medium, histidine was the sole source of nitrogen. In the remaining media the nitrogen source was $(NH_4)_2SO_4$. Exponentially growing cells were used and extracts prepared by ultrasonic disruption. Values are expressed as munits/mg of protein and are the average of three determinations from separate cultures.

	Specific activities (munits/mg of protein)				
Carbon source	Histidine–2-oxoglutarate aminotransferase	Histidase	Urocanase	Formiminoglutamate hydrolase	Formylglutamate hydrolase
L-Histidine	300	120	54	112	704
Urocanate	150	60	25	86	580
Imidazolylpropionate	190	65	26	92	568
Imidazolyl-L-lactate	280	210	41	115	730
L-Histidine+succinate	172	110	36	83	490
N-Formimino-L-glutamate		5	2	121	720
N-Formyl-L-glutamate		5	2	4	805
L-Glutamate	12	6	1	0	7
Succinate	15	7	1	0	7

were also not assayed because of the ambiguity as to which is the key enzyme involved in this step. Table 1 shows the specific activities of the enzymes concerned with histidine metabolism after growth of the organism on various substrates.

The results in Table 1 show that the specific activity of a particular enzyme is dependent upon the nature of the growth substrate even in cases where significant induction has occurred. All the enzymes are not affected equally, since, for example, the ratio of histidase/urocanase activity varies from approximately 5:1 with imidazolyl-L-lactate as the growth substrate to only 2.4:1 where cells are grown with urocanate. It has been shown in *K. aerogenes* (Magasanik *et al.*, 1965) that the ratio of histidase/urocanase activity remains constant over a wide range of absolute activities, and from this it has been concluded that the induction of the two enzymes is coordinately controlled. The clear variation of the histidase/urocanase activity ratio shown in Table 1 prompted a further investigation of the activities of these two enzymes under growth conditions leading to a wider variation in the extent of induction or repression. L-Histidine–2-oxoglutarate activity was also studied to see how this related to the activities of histidase and urocanase. Enzyme activities obtained from a large number of different cultures are shown in Figs. 1 and 2.

Fig. 1 shows a fairly wide scatter of points for the plot of urocanase activity against histidase activity. A good proportionality was maintained for any one growth condition, but relatively more histidase activity was produced during growth on imidazolyl-L-lactate, and to a lesser extent on L-histidine+succinate and on L-histidine alone, than on the other growth substrates.

A relatively good proportionality was maintained between the activities of the aminotransferase and urocanase under all conditions (Fig. 2), but this was not so with the aminotransferase and histidase, where again the constant ratio was disturbed by the greater histidase activities from cells grown with imidazolyl-L-lactate, L-histidine+succinate, or L-histidine alone.

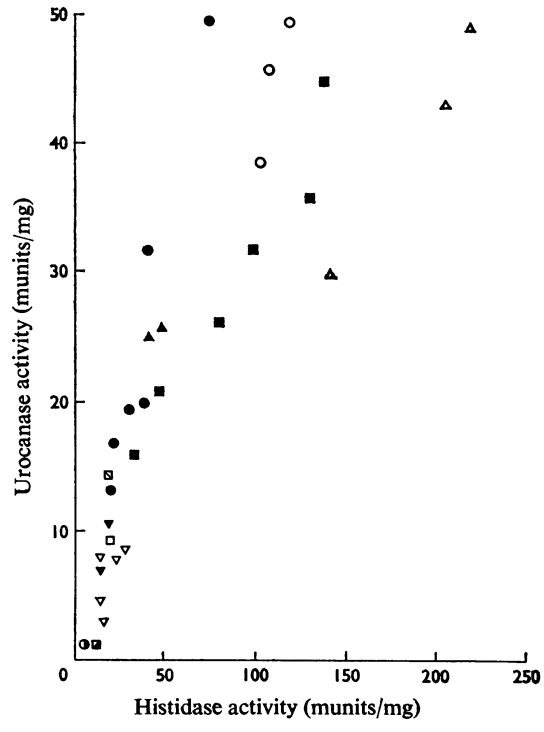


Fig. 1. *Relationship between the specific activity of urocanase and that of histidase in cell-free extracts*

Cells were grown in different media containing $(NH_4)_2SO_4$ as the nitrogen source and the following compounds as carbon source: ○, L-histidine; ●, urocanate; △, imidazolyl-L-lactate; ▲, imidazolyl-propionate; □, urocanate+succinate; ▧ imidazolyl-L-lactate+succinate; ▽, urocanate+L-glutamate; ▼, L-histidine+succinate; ◪ succinate; ◑ L-glutamate. One medium (■) contained L-histidine+succinate and no $(NH_4)_2SO_4$. Each point on the graph represents a single culture of exponentially growing cells. In some instances, cells were first grown overnight in the succinate medium and then transferred to a medium of the composition shown. They were then harvested when the cell mass had increased at least fourfold in the new medium.

Catabolite repression of the enzymes degrading L-*histidine*

The term 'catabolite repression' was used by Magasanik (1961) to describe the repression of synthesis of certain inducible enzymes when various carbon compounds are added to a culture in the presence of the inducer. Table 2 shows the effect of adding L-glutamate or succinate to the growth medium, and the effect of varying the available nitrogen source, on the induction of the enzymes that degrade L-histidine. L-Glutamate repressed the enzymes of the pathway to a greater extent than succinate, possibly because it was the immediate end product of the pathway. However, the growth of the organism on L-glutamate as sole carbon source was slightly faster than when succinate was used. The greater repression of the enzymes by L-glutamate is therefore in agreement with the work of Lessie & Niedhardt (1967), who showed that with *P. aeruginosa* the efficiency of a catabolite repressor was related to its ability to promote rapid growth.

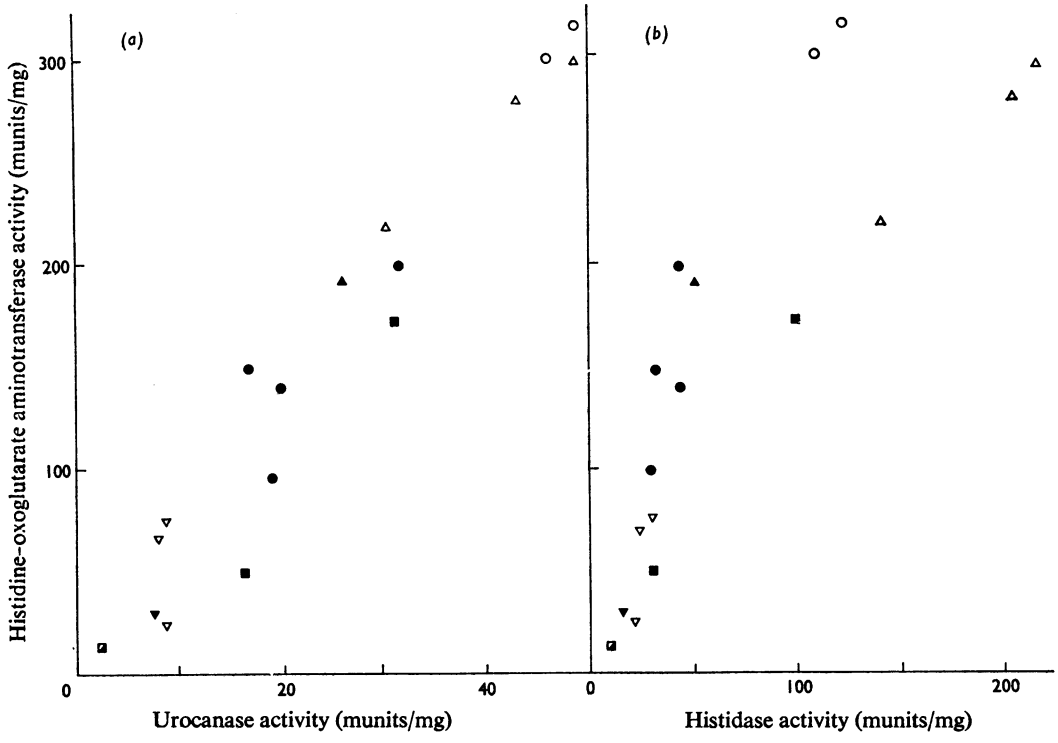


Fig. 2. *Relationship between the specific activity of* L-*histidine–2-oxoglutarate aminotransferase and that of* (*a*) *urocanase and* (*b*) *histidase*

The preparations and symbols used were those described in the legend to Fig. 1.

Table 2. *Catabolite repression of the enzymes degrading* L-*histidine in P. testosteroni*

All the growth media contained $(NH_4)_2SO_4$ as the nitrogen source except where indicated. The values for urocanate and L-histidine+succinate [minus $(NH_4)_2SO_4$] are taken from Table 1 and are included for convenience as representative of the fully induced state. Other conditions were as given in Table 1.

	Specific activity (munits/mg of protein)				
Growth medium	Histidine–2-oxoglutarate aminotransferase	Histidase	Urocanase	Formiminoglutamate hydrolase	Formylglutamate hydrolase
Urocanate	150	60	25	86	580
Urocanate+succinate	—	18	9	20	95
Urocanate+L-glutamate	21	16	6	9	56
L-Histidine+succinate	33	21	8	16	64
L-Histidine+succinate [minus $(NH_4)_2SO_4$]	172	110	36	83	490

Endogenous induction of enzymes of the histidine pathway in urocanase⁻ mutants

Table 1 shows that cells grown with imidazolylpropionate or urocanate as the carbon source had induced activities of the enzymes for degrading L-histidine. Yet of these, histidase and the aminotransferase are not required for the metabolism of the growth substrates. This fact, and the apparent coordinate synthesis of histidase, urocanase and the

aminotransferase (Figs. 1 and 2), suggested that these enzymes were induced by the first common intermediate of the breakdown pathway of the imidazole derivatives, i.e. urocanate. Evidence for this was obtained by studying enzyme activities in a mutant (A26) lacking the ability to synthesize active urocanase and another (11/13) which had a low activity of inducible histidase (Table 3). The results show that the urocanase$^-$ mutant, in the absence of inducer, has higher than basal, wild-type activities of the pathway enzymes. These results are similar to those obtained by Schlesinger *et al.* (1965) with *K. aerogenes* and Lessie & Neidhardt (1967) with *P. aeruginosa*, who showed that urocanase$^-$ mutants had constitutive activities of histidase and formiminoglutamate hydrolase. However, the results given here demonstrate that, in *P. testosteroni*, the endogenous induction is extended to include both the aminotransferase and formylglutamate hydrolase. The explanation of the constitutive enzyme synthesis in urocanase$^-$ mutants (Schlesinger *et al.*, 1965) is that urocanate is the inducer for the enzymes concerned and that sufficient of it is produced by the basal amount of histidase (and accumulated because of the block) to induce synthesis. The induction of formiminoglutamate hydrolase and formylglutamate hydrolase by the urocanase$^-$ mutant shows that the presence of the substrates for these enzymes is not essential for their synthesis to occur. Nevertheless, in the urocanase$^-$ mutant, both the constitutive activity of formylglutamate hydrolase and the fully induced activity obtained are low when compared with the maximum activity obtainable in the wild-type. This is best illustrated by the activities of the various enzymes in mutant A26 grown with histidine+succinate (Table 3). Histidase, the aminotransferase and formiminoglutamate hydrolase are induced to activities that exceed those of the wild-type grown in medium of the same composition, but formylglutamate hydrolase is induced to less than one-half of the wild-type value. Also, the ratio of formylglutamate hydrolase activity to histidase activity is much lower in mutant A26 grown on urocanate+glutamate than it is in the wild-type grown on urocanate. Whereas histidase activity is higher in the mutant than in the wild-type, formylglutamate hydrolase is considerably lower at some 14% of wild-type activity. Again this shows either the decreased response of the induction of formylglutamate hydrolase to urocanate or the increased sensitivity of its synthesis to catabolite repression.

Specificity of the transport system for imidazolylpropionate

The results reported in the preceding paper (Coote & Hassall, 1973) showed that cells, after growth with urocanate, could accumulate imidazolylpropionate but not oxidize it. The specificity of this transport mechanism was investigated by challenging cells that had accumulated [^{14}C]imidazolylpropionate with various intermediates of the pathway. The results are shown in Fig. 3. Both unlabelled imidazolylpropionate and urocanate brought about a rapid decrease in the amount of [^{14}C]imidazolylpropionate that had been accumulated, indicating a common transport mechanism. Imidazolyl-L-lactate also brought about the release of radioactivity, but at a slower rate, suggesting that this compound too is transported by the same system. These relative activities of the transport system toward imidazolylpropionate, urocanate and imidazolyl-L-lactate are to a large extent reflected in the relative rates of growth of the organism on these compounds. Neither L-histidine nor L-glutamate displaced [^{14}C]imidazolylpropionate from whole cells. The results presented in Table 4 show

Table 3. *Enzyme activities in mutants of P. testosteroni*

Conditions were as described in Table 1. Values (munits/mg) are the average of at least two determinations from separate cultures. Values in parentheses are activities obtained with the wild-type organism grown in media of the same composition. The L-histidine+succinate medium contained L-histidine as sole source of nitrogen.

	Specific activity (munits/mg)				
Mutant and carbon source	Histidine–2-oxoglutarate aminotransferase	Histidase	Urocanase	Formiminoglutamate hydrolase	Formylglutamate hydrolase
Mutant A26 (urocanase$^-$)					
L-Histidine+succinate	290 (172)	195 (110)	—	114 (83)	218 (490)
Succinate	60 (15)	34 (7)	—	12 (0)	52 (7)
L-Glutamate	48 (12)	23 (6)	—	7 (0)	39 (7)
Succinate+urocanate		93 (18)	—	76 (20)	156 (95)
L-Glutamate+urocanate		84 (16)	—	57 (9)	82 (56)
Mutant 11/13 ('leaky' histidase$^-$)					
Urocanate	168 (150)	4 (60)	28 (25)		

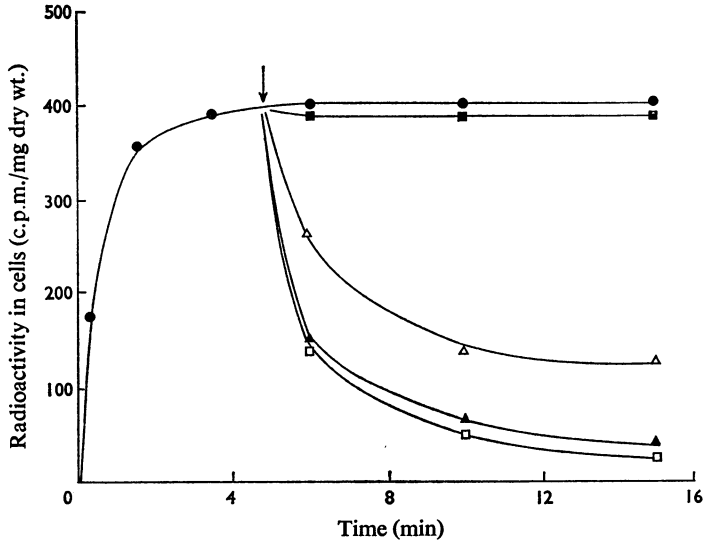


Fig. 3. *Uptake and displacement of [^{14}C]imidazolylpropionate from whole cells of the wild-type organism previously grown with urocanate as the source of carbon*

The incubation mixture, at 30°C, contained 0.02μmol of [^{14}C]imidazolylpropionate (0.01μCi), 0.5mg of chloramphenicol and 100μmol of KH_2PO_4 buffer (pH7.0) in 5ml. The reaction was started by the addition of 5ml of cell suspension (1mg dry wt./ml) from a syringe and 1ml samples were withdrawn at the times shown (●). Unlabelled compounds (0.5μmol in 0.1ml) were added to parallel incubation mixtures after 5min (indicated by the arrow); ▲, imidazolylpropionate; □, urocanate; △, imidazolyl-L-lactate; ■, L-histidine.

Table 4. *Independent induction of the mechanism for imidazolylpropionate uptake and of the ability to oxidize imidazolylpropionate in whole cells of the wild-type organism*

Accumulation of [^{14}C]imidazolylpropionate by washed cells was followed as described in the legend to Fig. 3. In all cases, except where imidazolylpropionate had been the growth substrate, accumulation of radioactivity was at a maximum after approx. 5min and remained constant. When cells had been grown with imidazolylpropionate the accumulation rose to a maximum at 3.5min and then decreased; maximum values were recorded. Ability to oxidize imidazolylpropionate was measured manometrically by using 3.5–5mg dry wt. of cells, 100μmol of KH_2PO_4 buffer (pH7.0) and 5μmol of imidazolylpropionate in 2.8ml; rates are corrected for endogenous respiration. In all cases $(NH_4)_2SO_4$ was the nitrogen source during growth.

Carbon source during growth	Uptake of [^{14}C]imidazolylpropionate (c.p.m./mg dry wt.)	Oxidation of imidazolylpropionate (μl of O_2/h per mg dry wt.)
Imidazolylpropionate	360	92
Imidazolyl-L-lactate	435	3
L-Histidine	420	2
Urocanate	450	2
L-Glutamate	41	3

that the transport mechanism for imidazolylpropionate is induced separately from the enzyme or enzymes converting this compound into urocanate. The permease is induced after growth with imidazolylpropionate, histidine, urocanate, or imidazolyl-L-lactate as the carbon source, but ability to oxidize imidazolylpropionate is dependent on the previous exposure of cells to the substrate.

Table 5. *Induction of histidase and urocanase and the mechanism for imidazolylpropionate uptake in a mutant (13/18) unable to degrade imidazolylpropionate*

Cells were grown in media containing $(NH_4)_2SO_4$ (1g/litre) as the nitrogen source and with the carbon compounds (2g/litre) present as shown. Other conditions are given in Table 4.

Organism	Carbon compounds present during growth	Histidase activity (munits/mg)	Urocanase activity (munits/mg)	Uptake of [^{14}C]imidazolylpropionate (c.p.m./mg dry wt.)
Mutant 13/18	L-Glutamate	4	1	46
	L-Glutamate+imidazolylpropionate	6	1	50
	Urocanate	57	32	445
Revertant of 13/18	L-Glutamate+imidazolylpropionate	23	10	—
Wild-type	L-Glutamate+imidazolylpropionate	21	9	—

Inability of imidazolylpropionate to act as an inducer for enzymes of the histidine pathway

Since in a number of organisms imidazolylpropionate is a gratuitous inducer for the enzymes of the histidine pathway, it was desirable to investigate its properties as an inducer in *P. testosteroni*, an organism capable of using it as a source of carbon for growth. A mutant (13/18) that had lost the ability to utilize imidazolylpropionate (but not urocanate) as the growth substrate was used. This mutant was known, therefore, to have either a defective permease or to lack the enzyme metabolizing imidazolylpropionate. The latter was considered the more likely, since urocanate and imidazolylpropionate are carried into the cell by the same system. The ability of imidazolylpropionate to induce representative enzymes of the histidine pathway and the permease was examined in this mutant. The activities were compared with those obtained when glutamate or urocanate was the carbon source for growth. Histidase and urocanase activities in the wild-type and revertant organisms were determined after growth in the imidazolylpropionate+glutamate medium (Table 5). The results confirm that mutant 13/18 is blocked in the metabolism of imidazolylpropionate rather than in the uptake of this compound. They also strongly suggest that imidazolylpropionate is not an inducer either for the enzymes of the histidine pathway or for the permease. Only basal activities of these systems are detectable after growth of the mutant on glutamate in the presence of imidazolylpropionate. On the other hand, growth of the wild-type in the same medium, where the formation of urocanate can readily occur, results in the induction of significant activities of histidase and urocanase.

Discussion

The results presented show that those enzymes of the histidine-degradative pathway of *P. testosteroni* which were studied are all inducible. Under conditions of non-induction, such as growth with L-glutamate or succinate as the carbon source, distinct basal activities of histidase, urocanase, histidine–2-oxoglutarate aminotransferase and formylglutamate hydrolase could be detected. The synthesis of the enzymes in the presence of an inducer was markedly decreased by the availability of a more easily utilizable carbon source such as succinate or glutamate. However, when L-histidine was the sole source of nitrogen for the organism, succinate no longer acted as a repressor. Presumably the physiological repressor of the enzymes is a nitrogenous compound, which must be readily formed from the nitrogen source. When L-histidine is used as the sole nitrogen source, in the presence of succinate, it must be assumed that there is no accumulation of the repressor compound.

Urocanate is implicated as the physiological inducer of the enzymes studied, since in a mutant lacking urocanase, and in the absence of exogenous inducer, some synthesis of the enzymes occurred. This has been explained (Schlesinger *et al.*, 1965) as being caused by endogenously accumulated urocanate, and the extent of induction can be increased by the addition of urocanate. However, the induction of formylglutamate hydrolase by urocanate (in the urocanase$^-$ mutant) was low relative to the induction of the other enzymes. This partial response may be due to the presence in the organism of two enzymes that are able to hydrolyse formylglutamate, one inducible by urocanate and the other by formylglutamate; the synthesis of the first of these could occur co-ordinately with the synthesis of urocanase. There is some further evidence in support of the presence of two, isofunctional enzymes. First, cells grown on formylglutamate have high activities of formylglutamate hydrolase but only basal activities of the other enzymes in the pathway. Secondly, we have not been able to isolate mutants unable to grow at the expense

of formylglutamate while retaining the ability to utilize glutamate as a source of carbon.

It is possible that similar enzyme duplication exists with respect to formiminoglutamate hydrolase in that activity is induced by urocanate in urocanase$^-$ mutants and by formiminoglutamate (and independently of urocanase) in the wild-type. Induction of activity is therefore, caused by either urocanate or formiminoglutamate.

A control system of this kind, for overlapping catabolic pathways, exists in *Moraxella calcoacetica*, where separate pairs of enzymes are induced for the hydrolysis of 2-oxoadipate enol-lactone and for the cleavage of 2-oxoadipate, depending on whether these compounds are intermediates of degradation of protocatechuic acid or benzoic acid (Cánovas *et al.*, 1967).

In general, similarity exists between the control of L-histidine degradation in *P. testosteroni* and the control in *K. aerogenes*, *S. typhimurium* and *P. aeruginosa*, where urocanate has been shown to be the inducer of histidase, urocanase and formiminoglutamate hydrolase. The results presented here carry the work further by implicating urocanate as the natural inducer for two other enzymes, L-histidine–2-oxoglutarate aminotransferase and formylglutamate hydrolase.

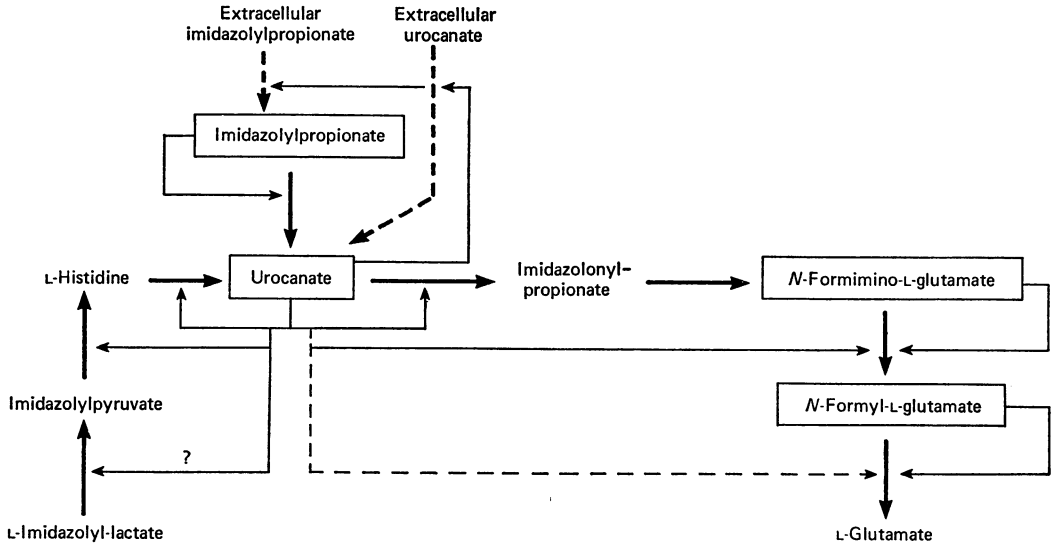
The expression of structural genes belonging to a single operon is quantitatively co-induced or co-repressed (Ames & Garry, 1959; Jacob & Monod, 1961). In these and other cases where the co-ordinate synthesis of enzymes was studied by comparing their activities, when cultures were subjected to various degrees of induction and repression, there was a scatter of points even though there was an obvious tendency for the enzymes to be produced in constant ratios to each other (Magasanik *et al.*, 1965; Ornston, 1966). Similar results were obtained in the work described here. A roughly constant ratio was maintained between the activity of urocanase and that of the aminotransferase under all conditions. Constant activity ratios were not obtained for histidase and the other two enzymes when they were similarly compared. Relatively higher histidase activities were obtained from cells grown with imidazolyl-L-lactate or L-histidine+succinate, and to a lesser extent with L-histidine alone. There are a number of possible reasons that might account for this.

First, although urocanate may be the inducer for the three enzymes, they may not be regulated in a co-ordinate manner. This cannot be ruled out until a regulatory mutant is isolated which is constitutive for all the enzymes in the absence of an inducer.

Secondly, the activities of one or more of the enzymes may be directly affected by the growth conditions. Jensen & Neidhardt (1969) found that, if a biosynthetic restriction (L-arginine limitation for the growth of an L-arginine auxotroph) was placed on *K. aerogenes* by using L-histidine as sole carbon source for growth, there was initially total repression of histidase synthesis. This was caused, Jensen & Neidhardt (1969) suggested, by a build-up of repressor molecules derived from L-histidine. In addition, histidase already present in the cells exhibited a decreased intracellular activity. *P. testosteroni* grows only slowly at the expense of imidazolyl-L-lactate and L-histidine and this may lead to only a limited endogenous accumulation of repressors derived from L-glutamate. This could in turn allow overproduction of the enzymes of the pathway. In the absence of excess of substrates both urocanase and the aminotransferase may preferentially decay and cause the discrepancy in ratios between the activities of these enzymes and that of histidase. The results show that specific activities of the enzymes were comparatively higher in extracts from cells grown with imidazolyl-L-lactate or L-histidine, both of which promote only slow growth.

Thirdly, decreased functioning of histidase in whole cells, as observed in *P. aeruginosa* grown under anaerobic conditions in the presence of nitrate (Jensen & Neidhardt, 1969; Lessie & Neidhardt, 1967), suggests that there is intracellular regulation of the activity of the enzyme. This is supported in *P. testosteroni* by the fact that histidase and the aminotransferase were fully induced when the organism was grown with urocanate or imidazolylpropionate. Yet these enzymes are unnecessary for the metabolism of either of these substrates, and activity of the enzymes *in vivo* must be exceedingly low to prevent breakdown of endogenous L-histidine. Similarly, the urocanase-deficient mutant (A26) grew as fast as the wild-type with L-glutamate or succinate and, although it possessed constitutive activities of histidase, it accumulated no urocanate in the medium. This again indicates that the intracellular activity of histidase towards endogenous L-histidine was negligible. One explanation of this is that there may in some way be physical separation of degradative and biosynthetic enzymes within the cell, so that L-histidine synthesized for protein manufacture is never allowed to approach enzymes whose function is purely degradative. Alternatively, histidase may be allosterically regulated and show a co-operative response with respect to substrate concentration. The sigmoidal saturation curves obtained for histidase from *P. aeruginosa* (Lessie & Niedhardt, 1967) and noted for the enzyme from *P. testosteroni* indicate that this is a possibility. There would thus be little activity of the enzyme at low concentrations of L-histidine.

Hug *et al.* (1968) have implicated succinate as a negative feedback inhibitor of the L-histidine-degradative pathway in *Pseudomonas putida*. They suggested that succinate inhibited urocanase and that the accumulated urocanate then inhibited histidase. However, it is difficult to envisage a sensitive indirect



Scheme 1. *Proposed control pattern for the induction of the enzymes degrading* L-*histidine and related compounds in P. testosteroni*

Degradation is shown by unbroken bold arrows and the common transport mechanism for urocanate, imidazolylpropionate and imidazolyl-lactate by broken bold arrows. Boxed metabolites are inducers for the steps indicated by light arrows. The broken light arrow shows partial induction and the query (?) shows that incomplete information is available.

feedback control of this kind. Physiologically speaking, it would seem somewhat paradoxical for negative feedback control to be effected by the build-up of a compound that was the inducer for the pathway being inhibited.

The pattern of control that emerges from the results presented here and to some extent in the preceding paper (Coote & Hassall, 1973) is shown in Scheme 1. This illustrates the central role played by urocanate in the induction of all the enzymes for metabolism of imidazolyl-lactate and L-histidine and in the induction of the common transport system for the uptake of urocanate, imidazolylpropionate and imidazolyl-lactate. The only enzyme (or enzyme system) not induced by urocanate is that which metabolizes imidazolylpropionate. The irreversibility of the histidase reaction (Peterkofsky, 1962) makes it unlikely that urocanate acts as an inducer by first being converted into histidine. However, whether or not histidine is also an inducer for the amino-transferase, histidase and urocanase could not be checked. The histidase activity in the 'leaky' histidase$^-$ mutant was such that any added histidine formed sufficient urocanate to bring about induction of the enzymes.

Genetic analysis of the structural genes for the enzymes degrading L-histidine in *B. subtilis* has shown that they are arranged in the order of the sequence of the enzymic reactions (Kimhi & Magasanik, 1970). Kaminskas & Magasanik (1970) presented evidence that these genes transcribe a polycistronic message and therefore constitute an operon. Our results, which indicate co-ordinate synthesis of histidase, urocanase and the amino-transferase, suggest that these enzymes could also constitute part of an operon. This might be extended to eight structural genes by the possible inclusion of the genes coding for imidazolonylpropionate amido-hydrolase, the transport protein for urocanate, a formiminoglutamate hydrolase, a formylglutamate hydrolase and an enzyme forming imidazolyl-pyruvate from imidazolyl-lactate. In contrast to what has been found with most of the other organisms studied, in *P. testosteroni* imidazolylpropionate does not appear to be an inducer *per se* for either the permease transporting it into the cell or for the enzymes of the histidine pathway.

We thank the Science Research Council for financial support.

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