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Induction of Histidine-Degrading Enzymes in *Pseudomonas aeruginosa*

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Urocanate but not histidine was able to induce formation of histidine-degrading enzymes in a histidine ammonia-lyase-deficient mutant of *Pseudomonas aeruginosa*. The results, in conjunction with others reported previously, indicate that urocanate, the first intermediate, is the physiological inducer of the pathway.

Previous studies of enzymes involved in histidine degradation by *Pseudomonas aeruginosa* indicated that enzymes I, II, IV of the pathway are induced in the presence of histidine or urocanate (2). Urocanate did not satisfy the histidine requirement of a histidine auxotroph; it served as an inducer without conversion to histidine. Enzymes I and IV were formed constitutively in a urocanase (enzyme II)-deficient strain, presumably as a consequence of the presence of urocanate accumulated through the action of basal levels of histidine ammonia-lyase on the internal pool of histidine. The results were similar to those found earlier for induction of histidine-degrading enzymes in *Aerobacter aerogenes* (4). However, studies of the histidine-degrading enzymes in *Bacillus subtilis* revealed that histidine rather than urocanate is the physiological inducer in this organism (1). Recently we isolated a histidine ammonia-lyase (EC 4.3.1.3)-deficient strain of *P. aeruginosa*. This enabled us to test whether histidine can serve as an inducer of histidine-degrading enzymes in *P. aeruginosa* without conversion to urocanate.

Strain CN15, the histidine ammonia-lyase-deficient mutant, was obtained from the wild type ATCC 7700 by selecting for bacteria unable to utilize histidine as sole carbon source. A modification of the well-known penicillin selection technique was used in which D-cycloserine was substituted for penicillin (3). Strain CN15 grew as rapidly as the wild type in media containing urocanate or citrate as carbon source. As indicated in Table 1, extracts of strain CN15 prepared from cells grown in media with 0.3% urocanate were deficient in histidine ammonia-lyase activity compared to extracts of strain 7700 grown under similar conditions. The level of urocanase (enzyme II) in the CN15 extracts was slightly lower than with strain 7700. The level of formiminoglutamate hydrolase (enzyme IV) was the same in the two strains.

TABLE 1. *Activities of histidine-degrading enzymes in extracts of strains CN15 and 7700 grown with urocanate*[a]

Strain	Specific activity[b]					
	I		II		IV	
	Absolute	Relative[c]	Absolute	Relative	Absolute	Relative
7700	0.485	100	0.149	100	0.181	100
CN15	0.007	1	0.110	73	0.193	107

[a] Growth of the bacteria and preparation of extracts as well as methods used to determine enzyme activities were as reported previously (1) with the following exception. Sonic disruption of the bacterial suspensions was for 1 min with a Bronwill 20-kc probe sonifier operated at 80% full power. The suspensions were kept in an ice bath during this treatment.

[b] Expressed as micromoles of urocanate formed or consumed per minute per milligram of protein for enzymes I and II or micromoles of formiminoglutamate consumed per minute per milligram of protein for enzyme IV.

[c] Defined as per cent activity relative to strain 7700 grown with urocanate.

To test whether histidine can induce formation of histidine-degrading enzymes without conversion to urocanate, strain CN15 was grown in citrate medium supplemented with 0.1% L-histidine, and the levels of enzymes II and IV of the pathway were compared with those in cultures of strain 7700 grown under the same conditions (Table 2). A similar comparison was carried out with bacteria grown in citrate media supplemented with 0.1% urocanate. The results show

that the enzymes were formed in the presence of urocanate but not in the presence of histidine, indicating that urocanate but not histidine is the inducer. In strain CN15, urocanase was considerably less sensitive to catabolite repression promoted by the presence of citrate than it was in the wild type. If this is a consequence of the loss of histidine ammonia-lyase activity, the results would suggest a direct role of this enzyme in pathway repression.

To rule out the possibility that a defective uptake system might be a primary cause of the inability of strain CN15 to utilize histidine, the capacity of strain CN15 to take up histidine from the medium was compared with that of the wild type. Strains CN15 and 7700 were grown in citrate medium supplemented with histidine, and then transferred to similar medium containing ^{14}C-L-histidine. The incorporation of histidine into cold trichloroacetic acid -soluble and -insoluble fractions of the bacteria was found to be the same with both strains (Fig. 1A and 1B). The

TABLE 2. *Comparison of the abilities of histidine and urocanate to induce formation of histidine-degrading enzymes in strains CN15 and 7700*

Additions to basal salts medium	Strain	Specific activity[a]			
		II		IV	
		Absolute	Relative[b]	Absolute	Relative
0.3% Citric acid	7700	0.026	17	0.166	92
+					
0.1% L-Histidine	CN15		<0.01	0.002	1
0.3% Citric acid	7700	0.023	15	0.160	89
+					
0.1% Urocanic acid	CN15	0.046	32	0.156	86

[a] See footnote *b*, Table 1.
[b] See footnote *c*, Table 1.

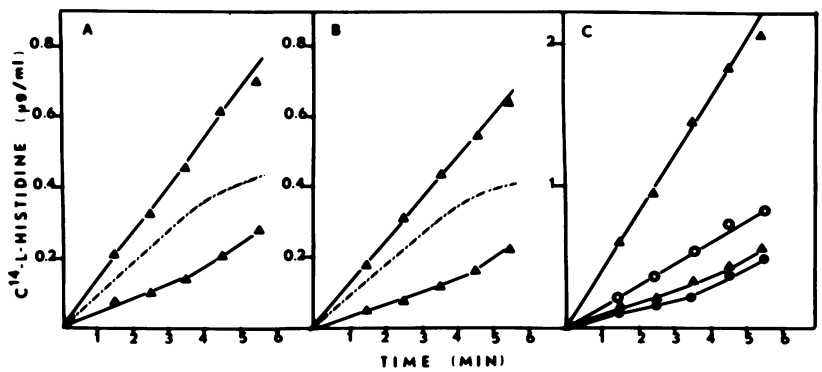

FIG. 1. *Incorporation of ^{14}C-L-histidine into Pseudomonas aeruginosa strain CN15 and ATCC 7700. At zero time, cells were added to citrate medium containing 5 × 10^{-4} M L-histidine with approximately 10^3 counts/min of L-histidine per μg and incubated at 24 C. Results not shown indicated that the increase in rate of uptake with increasing concentration of histidine followed Michaelis-Menten kinetics, with a K_m of about 2 × 10^{-4} M for L-histidine. Samples (0.5 to 1.0 ml) containing 15 to 30 μg of protein/ml were removed at the indicated times, and the bacteria were collected by filtration. Cells retained on the filters were washed with warm medium without substrate (the cellular pool of histidine was expelled during washes with cold medium) or cold 5% trichloroacetic acid. The filters were dried, and radioactivity was determined in a scintillation counter. The history of the cells prior to the start of the experiment was as follows. (A) Strain 7700 grown in citrate medium supplemented with histidine. (B) Strain CN15 grown as in A. (C) Strain 7700: (▲, △) grown in medium containing 0.3% L-histidine; (●, ○) grown in medium containing 0.3% acid. Open symbols represent medium-washed cells, closed symbols represent trichloroacetic acid-washed cells, and dashed lines represent the acid-soluble fraction calculated from values for medium- and acid-washed bacteria.*

amount of histidine taken up into the acid-soluble pool over the 5-min period indicated represents approximately a 20-fold concentration of histidine over the amount initially in the medium. Addition of 10^{-2} M azide immediately abolished uptake of histidine, indicating that the process is energy-dependent.

Comparison of the rates of ^{14}C-histidine incorporation into bacteria grown with citrate and with histidine as carbon source indicated that the capacity of strain 7700 to take up histidine is not regulated coordinately with the enzymes of the histidine-degrading pathway. The results (Fig. 1C) show that growth of the bacteria with histidine increased the rate of uptake; however, the 2- to 3-fold difference in rate of histidine uptake between the histidine- and citrate-grown bacteria was small compared to the 10- to 50-fold difference in levels of histidine-degrading enzymes reported earlier.

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