INHIBITION OF HISTIDINE DEAMINASE BY L-TYROSINE AND P-HYDROXYPHENYLPYRUVATE

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Feedback inhibition as a method for metabolic control has not been found often in catabolic pathways. Regulatory interactions ("cross inhibition") between pathways were predicted by Jacob and Monod (1961). In this investigation histidine deaminase was shown to be inhibited by metabolites of another pathway: L-tyrosine and p-hydroxyphenylpyruvate. The inhibition exhibits competitive inhibition kinetics. The inhibition is pH dependent, is antagonized by zinc, and is diminished by added glutathione.

Histidine degradation and the regulation of the pathway has been studied in bacteria and the literature is summarized by Lessie and Neidhardt (1967). The histidine pathway was shown to be inducible and subject to catabolite repression. Lessie and Neidhardt (1967) suggested that histidine deaminase may be an allosteric control enzyme since sigmoid saturation curves were obtained in pyrophosphate buffer. However, they observed no inhibition when 16 potential "feedback" inhibitors were tried.

METHODS

Pseudomonas putida A 3•12 was grown on L-histidine at room temperature in shake flasks in the medium of Tabor and Mehler (1955). The harvested cells were washed three times in .01 M K-PO_4 buffer, pH 7.0. Cells were ruptured in the Hughes press and diluted to 10 volumes in 0.01 M K-PO_4 buffer, pH 7.0. The extract was treated with DNase (2 μg/ml) for 15 min. at 25°C to reduce viscosity. Urocanase was inactivated by heat treatment of the supernate 78-83°C for 15 min. and centrifugation 20 min. at 12,000 x g at 4°C.

Further purification steps with added glutathione resulted in loss of the enzyme or enzyme form which is sensitive to the inhibitor. The assay is based on the method of Tabor and Mehler (1955). The cuvette contained (except where indicated): 2 μmoles L-histidine, 100 μmoles K-PO_4, pH 7.8, .05 ml crude enzyme (0.2 mg protein), total volume, 3.0 ml. Inhibitor and glutathione were added as indicated elsewhere and were incubated with enzyme and buffer 10 min. at 30°C before the reaction was started by addition of histidine. A plot of enzyme concentration versus initial velocity was linear.

RESULTS AND DISCUSSION

L-Tyrosine inhibits freshly prepared enzyme. Fig. 1a shows data of an experiment plotted in the reciprocal Lineweaver-Burk form. The curves are typical of competitive inhibition kinetics. Tyrosine concentration in this experiment was 1.7 mM. The K_I for tyrosine was calculated using the data from curves like Fig. 1. Seven values were averaged for a K_I of 1.9 mM. The K_m for histidine was 2 mM.

The inhibition of histidine deaminase by p-hydroxyphenylpyruvate is shown by data given in Fig. 1b. This metabolite is related to L-tyrosine by transamination in both biosynthesis and degradation. This is the Lineweaver-Burk plot, and this too indicates competitive inhibition. The K_I was calculated from four of these curves and averaged to give .04 mM. These experiments were run at pH 7.8 which was a compromise between high enzyme activity and high inhibitory activity. As Stadtman discussed in his review (1966), "so-called" competitive inhibition as revealed by reciprocal plots is not necessarily characterized by actual competition of substrate and inhibitor for the catalytic site. Thus, competitive inhibition kinetics do not rule out two sites.

The experiment shown in Fig. 2a shows the effect of tyrosine concentration on the percent inhibition. Unexpectedly these data when plotted gave a complex curve. Sigmoid curves are often characteristic of allosteric

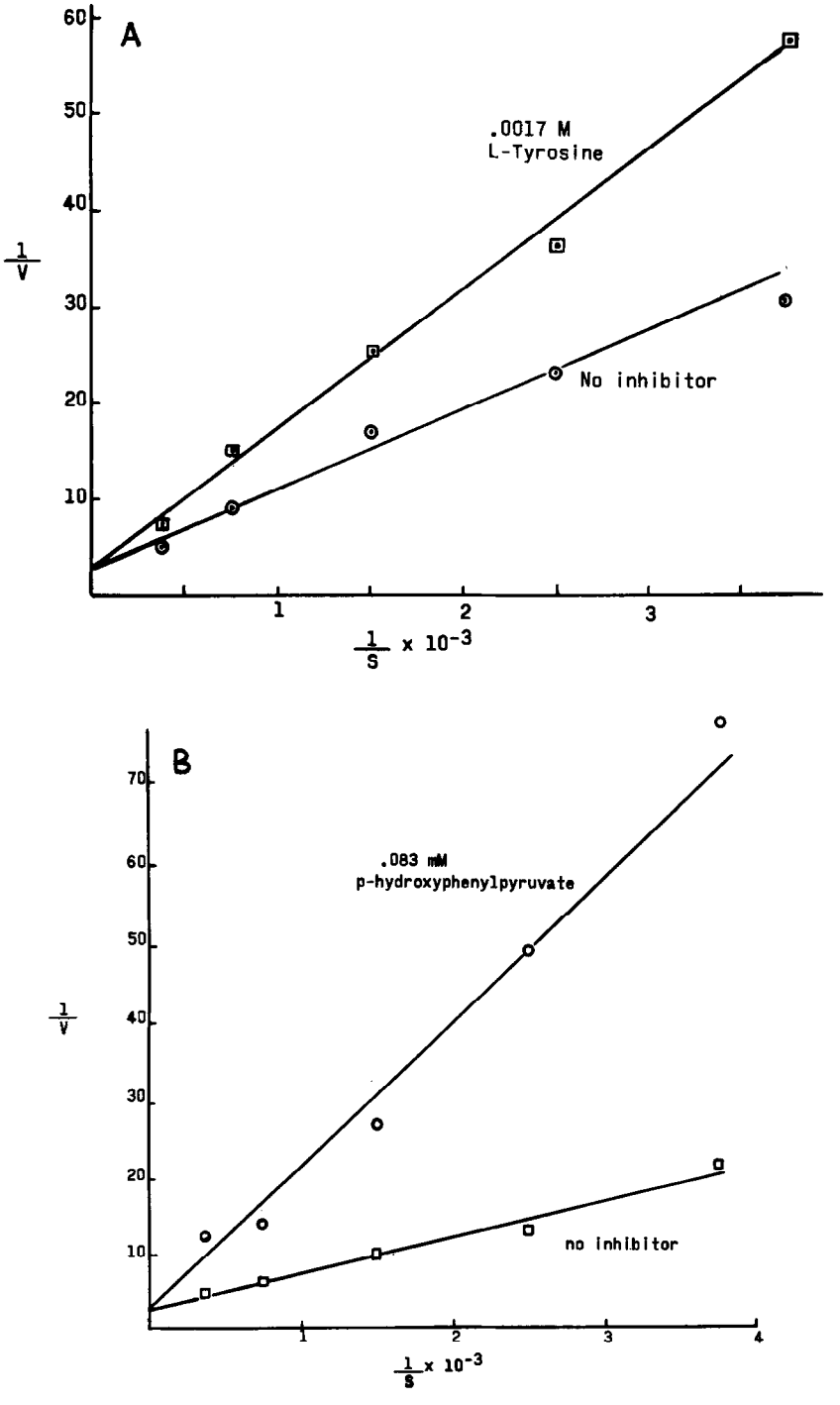

FIG. 1. Double-reciprocal plot of reaction velocity ($A_{277\ m\mu}$/min.) versus histidine concentration. Reactions run as described in Methods. (a). Inhibitor L-tyrosine, 1.7 mM final concentration. (b). Inhibitor p-hydroxyphenylpyruvate, .083 mM final concentration.

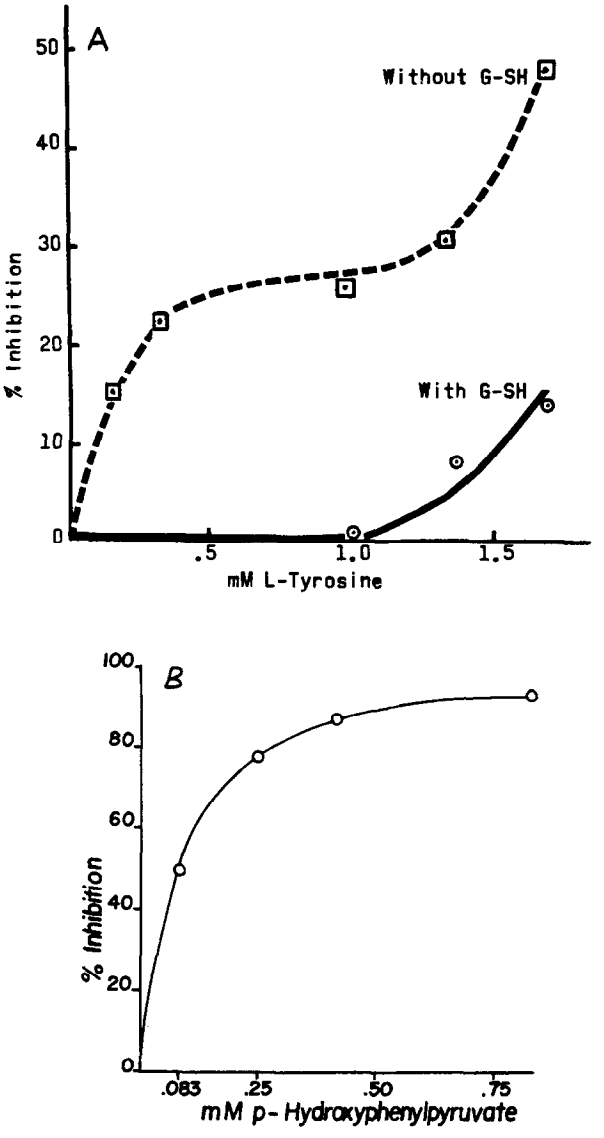

FIG. 2. Effect of inhibitor concentration on percent inhibition of histidine deaminase. Reactions run as described in Methods. (a). Inhibitor L-tyrosine; reduced glutathione .0167 M, final concentration. (b). Inhibitor, p-hydroxyphenylpyruvate.

enzymes (Stadtman, 1966). This experiment suggests that more than one form of histidine deaminase may be present. The shape of this curve has been reproduced ten times with ten enzyme preparations, although the numerical values have varied from those given in Fig. 2a. When reduced glutathione

(1.67 mM) was added to the cuvette and this "saturation" curve for tyrosine was determined, the percent inhibition was greatly reduced and the shape of the curve (Fig. 2a) was changed to what may be the first half of a sigmoid curve. This change was reproduced in eight experiments. Glutathione, which is usually added for this enzyme assay (Tabor and Mehler, 1955), apparently partially desensitizes the enzyme to tyrosine inhibition or converts the enzyme to a form which has different inhibition characteristics.

The saturation curve for inhibition by p-hydroxyphenylpyruvate is given in Fig. 2b. This curve is hyperbolic. This inhibitor is effective at much lower concentrations than tyrosine and does not exhibit the sigmoid or complex shape of the tyrosine curve. The addition of glutathione usually enhances the activity of the enzyme (depending on age, storage, etc.), but the addition of glutathione (1.67 mM) abolishes the inhibition by p-hydroxyphenylpyruvate at the inhibitor levels used in Fig. 2b.

Although these inhibitors are effective at pH 7.2 and 7.8, there is little or no inhibition when the assay is carried out at pH 8.5.

Eight divalent cations were tested for their ability to affect the reaction and to prevent the inhibition by p-hydroxyphenylpyruvate (Table I). Zn^{++} and Mg^{++} stimulated the reaction while Cu^{++} and Hg^{++} inhibited. Mn^{++} and Cu^{++} increased the inhibition by p-hydroxyphenylpyruvate, and Zn^{++} and perhaps Sr^{++} antagonized the inhibition. The assay system precipitated when each of the cations was added in amounts equal to the tyrosine inhibitor concentration (1.7 mM). There is evidence that histidine deaminase has a metal cofactor (Peterkofsky and Mehler, 1963) and the inhibitions described here may very likely involve the metal. Wyatt (1964) has proposed that allosteric feedback inhibition for metabolic regulation may occur by way of metal cofactors and natural metal binding agents.

The freshly prepared enzyme has high activity and on standing at 0°C continually loses activity. Glutathione can restore most but not all of this loss of activity. However, the glutathione-treated enzyme is less sensitive

TABLE I: The Effect of Cations on Histidine Deaminase and on the Inhibition by p-Hydroxyphenylpyruvate

Cation[1] Added	Control A[3]	+Inhibitor[2] B[3]	Ratio B/A
None	100	58	.58
$ZnSO_4$	170	152	.89
$SrCl_2$	99	77	.78
$BaCl_2$	107	60	.56
$CoCl_2$	104	53	.51
$MgCl_2$	126	60	.48
$CuCl_2$	49	8	.16
$MnCl_2$	105	10	.10
$HgCl_2$	11	--	---

1. Final concentration .067 mM, cation.
2. Final concentration .05 mM, p-hydroxyphenylpyruvate.
3. The standard assay system was used. Rates are relative.

to inhibition. These observations favor the proposal that histidine deaminase is an allosteric enzyme because a catalytically active enzyme shows little response to the inhibitor.

Cerutti and Guroff (1965) have shown in Pseudomonas that tyrosine acts as a "chemical signal" by stimulation of prephenate dehydratase to form phenylalanine. Phenylalanine acts as an end-product inhibitor on the same enzyme. This combination tends to bring phenylalanine levels in balance with tyrosine. To extend this idea, tyrosine (or indirectly via p-hydroxyphenylpyruvate) may act as a signal to block a degrading enzyme, histidine deaminase, and thus tyrosine tends to regulate the balance of amino acids in one more way. Histidine may in this way be spared for protein synthesis and not degraded.

REFERENCES

Cerutti, P., and Guroff, G., J. Biol. Chem., 240, 3034 (1965).
Lessie, T. G., and Neidhardt, F. C., J. Bacteriol., 93, 1800 (1967).
Monod, J., and Jacob, F., in "Cold Spring Harbor Symposia on Quantitative Biology, Cellular Regulator Mechanisms" (L. Frisch, ed.), Vol. XXVI, p. 391. Waverly Press, Baltimore (1961).
Peterkofsky, A., and Mehler, L. N., Biochim. Biophys. Acta, 73, 159 (1963).
Stadtman, E. R., Advan. Enzymol., 28, 41 (1966).
Tabor, H., and Mehler, A. H., in "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. II, p. 228. Academic Press, New York (1955).
Wyatt, H. V., J. Theoret. Biol., 6, 441 (1964).