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THE REGULATION OF MOUSE LIVER ORNITHINE DECARBOXYLASE BY METABOLITES

COLIN G.D. MORLEY and HELEN HO

Department of Medicine and the Pritzker School of Medicine, University of Chicago, 950 E. 59th St., Chicago, Ill. 60637 (U.S.A.)

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Summary

The enzyme ornithine decarboxylase (L-Ornithine carboxy-lyase, EC 4.1.1.17), has been partially purified from the livers of mice subjected to partial hepatectomy (6—8 h previously).

Mouse liver ornithine decarboxylase requires pyridoxal phosphate, and dithiothreitol for maximal activity. The enzyme has a pH optimum of 7.3, it is inhibited in the presence of 0.3 M phosphate, glycine, Tricine and Tris. It shows no dependence on metal ions and is inhibited by high salt concentrations, particularly ammonium salts.

The kinetics of the enzyme have been studied with putrescine (and analogs), spermidine and spermine, in the presence of both high and low levels of pyridoxal phosphate. High concentrations of pyridoxal phosphate inhibit the enzyme. The enzyme is also inhibited by low concentrations of putrescine (1 mM). As the concentration of putrescine increased to 10 mM, non-competitive inhibition was observed, this could be reversed by addition of higher levels of pyridoxal phosphate. Spermidine and spermine inhibit (noncompetitively) only at high concentrations (10 mM). Ornithine inhibits at high concentrations (2 mM).

Spectral studies have shown that the observed kinetics of competitive inhibition at low concentrations of polyamine changing to noncompetitive inhibition at high polyamine concentrations are due to competition between enzyme and substrate (or inhibitor) for free (non-enzyme bound) pyridoxal phosphate. Noncompetitive inhibition arises through the formation of transient Schiff base complexes between amines and free pyridoxal phosphate.

It also appears that the binding of substrate to the active site takes place through Schiff base formation with enzyme bound pyridoxal phosphate.

Introduction

The pyridoxal phosphate dependent enzyme, ornithine decarboxylase (L-Ornithine carboxylyase EC 4.1.1.17) which catalyzes the conversion of ornithine to putrescine (the first and probably rate-limiting step in polyamine biosynthesis [1]) has been studied in a number of tissues, including rat liver [2], rat prostate [3] and malignant tissue [4,5]. Tissue levels of the enzyme rise following treatment of animals with hormones [6—10], under stress [11], during tissue replacement or growth (as in liver regeneration [11—14]) and in tumors [4]. The in vivo increases in ornithine decarboxylase seem to reflect a de novo enzyme synthesis [15] (or possibly decreased enzyme destruction) since the stimulated increases can be blocked with cycloheximide or actinomycin D [16]. Ornithine decarboxylase also has a very short half life, of the order of 10—20 min, in liver [17].

As a result of the above and the apparent lack of low molecular-weight effectors [2,10] with the partially purified enzyme from rat ventral prostate or rat liver, it has been concluded that the activity of mammalian ornithine decarboxylase is regulated by the rate of synthesis and degradation of the protein. (A recent report by Janne and Holta [18], indicates that in vivo inhibition of ornithine decarboxylase in regenerating rat liver can be effected by treatment of the animal with putrescine, spermidine or spermine).

It is the intent of this report to demonstrate that regulation of pre-existing ornithine decarboxylase by putrescine, spermidine and spermine and co-factor (pyridoxal phosphate) availability does occur.

Materials and Methods

Animals. Male CF_1 mice, from seven weeks to three months old were used. The mice were housed and fed as described earlier [19]. A weight range of 25—30 g was usual for the animals.

Animal treatment. Partial hepatectomies were performed under ether anesthesia by the method of Higgins and Anderson [20]. Livers from groups of animals (20—30), were pooled as sources of the enzyme.

Materials. [1-^{14}C]ornithine · HCl (specific activity 4.62 Ci/mmol) was purchased from New England Nuclear, Boston, Mass. and was dissolved in water, acidified and evaporated to dryness on a rotary evaporator to remove free $^{14}CO_2$. The purity of [^{14}C]ornithine was shown to be 98% before use, using the phosphoric acid cellulose system of Kremzner [21]. Putrescine was also purified by this procedure before use. Dithiothreitol was from Nutritional Biochemicals and pyridoxal phosphate from Sigma, St. Louis, Mo. Putrescine was from Eastman Kodak, Rochester, N.Y. Other materials were from suppliers mentioned in the text or in earlier reports [22,23].

Methods. Liver cell extracts were obtained by homogenization of pooled livers in cold 15 mM potassium phosphate buffer, (pH 7.3) or 0.1 M Tricine containing 5 mM EDTA and 10 mM dithiothreitol.

Partial purification of the enzyme. Partial purification of the enzyme was achieved through the following procedure:

1. Liver homogenate was centrifuged for 10 min at 10 000 × g in a Sorvall RC2B centrifuge.

2. The supernatant solution was then centrifuged at 100 000 × *g* for 1 h on a Beckman LS ultracentrifuge.

3. The solution was then treated with solid ammonium sulfate to 75% saturation, stirred at 0° C for 15 min, then centrifuged. The precipitate was discarded.

4. The supernatant solution was dialyzed against 5 vols. of 0.1 M Tricine (pH 7.3)/5 mM EDTA, 10 mM dithiothreitol and 50 mM pyridoxal phosphate.

5. The supernatant solution was passed through a UM20 Amicon filter, under N_2 pressure; the retentate was used for step 6.

6. The UM20 retentate (100 mg protein) was applied to a 5 × 1.5 cm column for DEAE-Sephadex [2] and the enzyme eluted with the same buffer used in step 4 with the addition of 0.2 M NaCl [2].

An approximately 40-fold purification of the enzyme was achieved as determined by the increase in specific activity of the final extract relative to the supernatant solution of step 2 (Table I).

Additional purification was carried out on some samples of the enzyme for spectral studies (see legend to Fig. 1) (Table I) but the more purified material appeared to be unstable and activity was lost on storage (at 1—2 mg/ml protein concentration and in dithiothreitol) at —20° C.

Ornithine decarboxylase assays. The normal assay mixture was 2.5 mM dithiothreitol, 0.2 mM pyridoxal phosphate, 15 mM phosphate or 0.1 M Tricine (pH 7.3) and 0.2—0.3 mM [^{14}C]ornithine · HCl (0.1 μCi) in 1 ml total volume. An incubation period of 30 min at 37° C was routinely used. 1 ml of 2 M citric acid was added at the end of the incubation period to stop the reaction and release trapped $^{14}CO_2$. A further incubation of 30 min was then carried out to release all $^{14}CO_2$. The $^{14}CO_2$ was collected in disposable plastic troughs which contained a trapping agent consisting of ethylene glycol/ethanolamine (4 : 1, v/v). Under the assay conditions, the reaction was linear for up to 80 min at 37° C. The pH optimum for the reaction in phosphate buffer was 7.3. The buffer and its concentration proved critical with this enzyme. The buffers of choice were Tris or Tricine, the latter at 0.1 M was used in most of the experi-

TABLE I

PURIFICATION OF MOUSE LIVER ORNITHINE DECARBOXYLASE (ODC)

Step *	Specific activity nmol CO_2/h/mg protein	Protein (mg)	Yield of activity (%)	Range of purification factor **
100 000 × *g* supernatant	840	1038	100	
75—100% $(NH_4)_2SO_4$	3 678	271	57	3—4-fold
UM 20 retentate	9 870	108	21	8—10-fold
DEAE-Sephadex	21 100	10	12	30—40-fold
Sephadex G-100 ***	176 310	0.3	4	120—200-fold

* Details of each step are given in the text. The data are from a representative experiment.

** Purification factors are expressed as an experimental range and are related in each case to the activity of the 100 000 × *g* supernatant.

*** This step was only used for enzyme preparations employed in the spectral studies.

ments described here (phosphate, imidazole and glycine inhibit). The activity of the enzyme throughout is expressed as pmol $^{14}CO_2$/h/mg protein.

Other methods. Measurement of protein concentrations was by the Lowry method [24] using crystalline bovine serum albumin (Armor) as standard. Liquid scintillation counting was performed in "Aquasol" (New England Nuclear) with an "Isocap" 300 Nuclear Chicago scintillation counter.

Spectral data was obtained on a Zeiss PMQ using 1 ml quartz cuvettes with a 1 cm light path.

Data was processed by linear regression analysis to obtain the kinetic constants, K_m, K_I and V.

Results

Spectrum of the enzyme. Fig. 1 presents spectral data for the partially purified enzyme preparation, dialyzed enzyme, enzyme plus dithiothreitol and free pyridoxal phosphate. The peak at 325 nm and the broad maximum at 380—395 nm indicate the presence of the pyridoxal phosphate cofactor on the

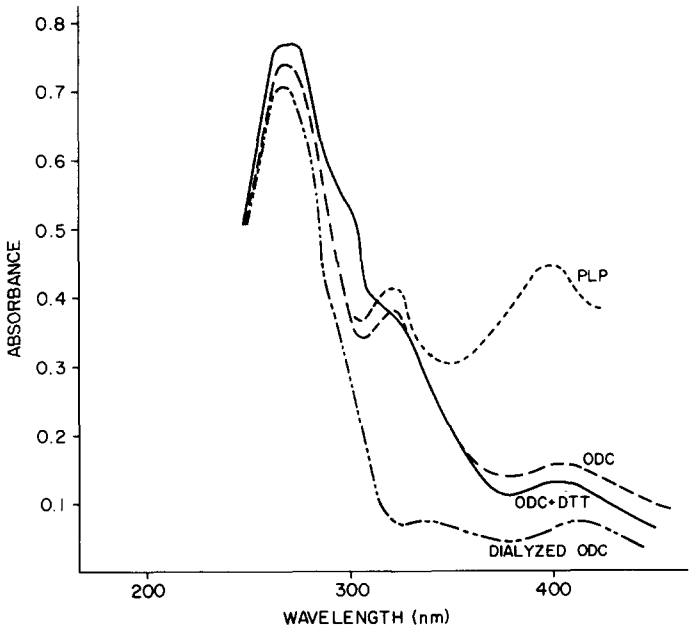

Fig. 1. Ultraviolet and visible spectral data for ornithine decarboxylase and pyridoxal phosphate at pH 7.2. 0.05 M sodium phosphate buffer (pH 7.2) was used. The enzyme preparation (70 mg protein) used for these studies was purified (following the DEAE Sephadex Step (see Methods) by passage over a Sephadex G-100 column (30 X 1 cm) in 0.1 M Tricine and 10 mM dithiothreitol. (pH 7.3). The fractions containing significant ornithine decarboxylase activity were pooled. Acrylamide gel electrophoresis showed one major protein band with a number of minor ones. Purification was approximately 200-fold (Table I) over the ornithine decarboxylase in the original 100 000 X *g* supernatant. ———, Spectrum of ornithine decarboxylase as isolated without additions. - - - - - -, Spectrum of free pyridoxal phosphate (PLP). (———) Spectrum of ornithine decarboxylase (ODC) and dithiothreitol (DTT) (2.5 mM). - - — - -, Spectrum of ornithine decarboxylase dialyzed free of pyridoxal phosphate.

enzyme. The slight shift in the maximum of the bound pyridoxal phosphate compared with the free pyridoxal phosphate could be attributed to the binding of the cofactor to the protein. Exhaustively dialyzed enzyme (three days with six buffer changes) shows only a 280 nm maximum. Addition of pyridoxal phosphate; and dithiothreitol followed by brief dialysis (24 h with two buffer changes) against dithiothreitol-containing buffer restores the full spectrum of enzyme.

Requirements for the enzyme. (Table II). Exhaustively dialyzed enzyme required added pyridoxal phosphate and dithiothreitol for its activity. There is apparently no metal ion requirement. Glutathione (2 mM) was unable to substitute for dithiothreitol.

Ornithine decarboxylase from mouse liver is inhibited by semicarbazide · HCl and ammonium salts.

Substrate saturation of ornithine decarboxylase. Fig. 2 shows the effect of increasing the concentration of ornithine from 0 to 200 μM. Saturation type kinetics are observed in this range of concentrations with a calculated K_m for ornithine of about 26 μM. At higher ornithine concentrations (2—4 mM) there is a sharp drop in activity (substrate inhibition) (Fig. 2).

The effect of increasing pyridoxal phosphate on ornithine decarboxylase activity. Increasing levels of pyridoxal phosphate at constant substrate (0.3 mM) increased the activity of dialyzed enzyme until 0.25 mM pyridoxal phosphate was reached. Above this level, inhibition of activity occurred. Analysis of this effect is shown in Fig. 3 in the Lineweaver-Burk and Eadie-Hofstee plots for ornithine decarboxylase activity at two levels of pyridoxal phosphate (0.25 mM and 3 mM). As the concentration of pyridoxal phosphate is raised above the optimum (0.25 mM), the V for the reaction is about doubled, but there is a large increase in the K_m for ornithine (from 26 to 351 μm). The apparent K_m for pyridoxal phosphate is 0.1 mM (Fig. 4).

TABLE II

REQUIREMENTS FOR ORNITHINE DECARBOXYLASE ACTIVITY

Conditions	Ornithine decarboxylase activity (pmol CO_2/mg/30′) × 10^{-2}
Dialyzed enzyme *	8 ± 2
Dialyzed enzyme + pyridoxal phosphate (0.25 mM)	35 ± 8
Dialyzed enzyme + dithiothreitol (2.5 mM)	12 ± 3
Dialyzed enzyme + pyridoxal phosphate + dithiothreitol	76 ± 10
Dialyzed enzyme + pyridoxal phosphate + glutathione (1 mM)	10 ± 3
Complete system ** + EDTA (1 mM)	72 ± 8
Complete system + Mg^{2+} (1 mM)	78 ± 6
Complete system + Semicarbazide · HCl (1 mM)	7 ± 1
Complete system + NaCl (100 mM)	60 ± 9
Complete system + NH_4Cl (100 mM)	18 ± 8

* Partially purified ornithine decarboxylase was dialyzed against 1 mM EDTA, in 0.1 M Tricine (pH 7.3) for 24 h, with 2 changes of buffer.

** "Complete system" is dialyzed enzyme + pyridoxal phosphate (0.25 mM) + dithiothreitol (2.5 mM) + 0.1 M Tricine (pH 7.3).

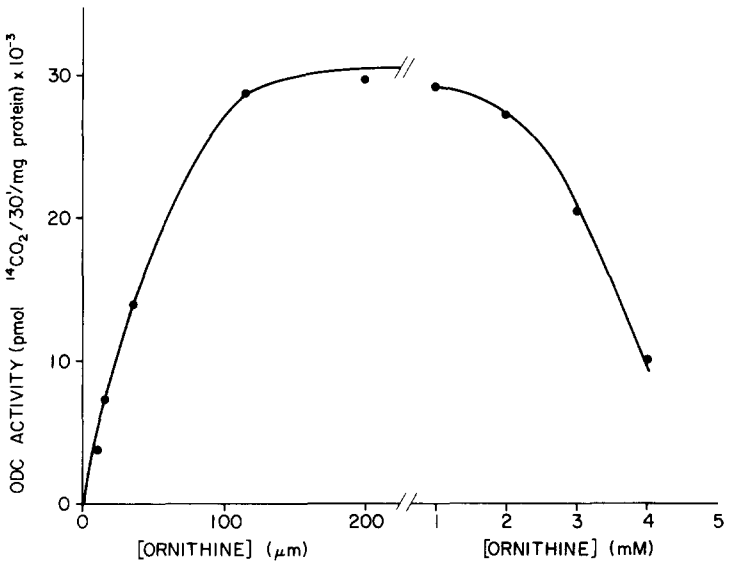

Fig. 2. Substrate saturation curve for ornithine decarboxylase. Ornithine concentrations of 0 to 200 μM and from 1 to 4 mM (note different abscissa scale for latter) were employed.

The effect of putrescine on ornithine decarboxylase activity. Fig. 5 presents kinetic data for putrescine inhibition of the enzyme, at both low and high concentrations of pyridoxal phosphate. At 0.25 mM pyridoxal phosphate, and moderate concentrations of putrescine (2.5 mM), almost classic competive inhibition is seen (Fig. 5A). At higher levels of putrescine (10 mM), the kinetics become more complex. There is a marked reduction in the V ($23 \cdot 10^3$ to

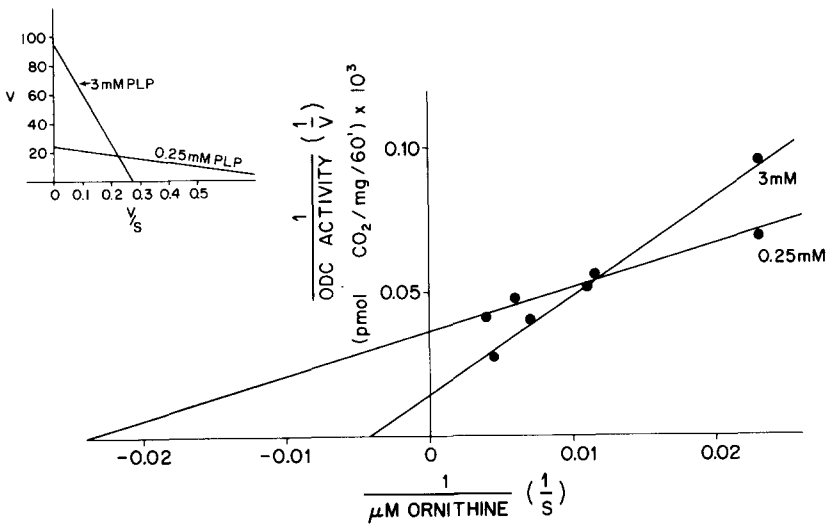

Fig. 3. The effect of pyridoxal phosphate (PLP) on ornithine decarboxylase kinetics. Insets in this and Figs. 4 and 5 are Eadie-Hofstee kinetic plots of the same data presented in the Lineweaver-Burk graphs. Pyridoxal phosphate (PLP) was tested at 2 concentrations, 0.25 mM and 3 mM.

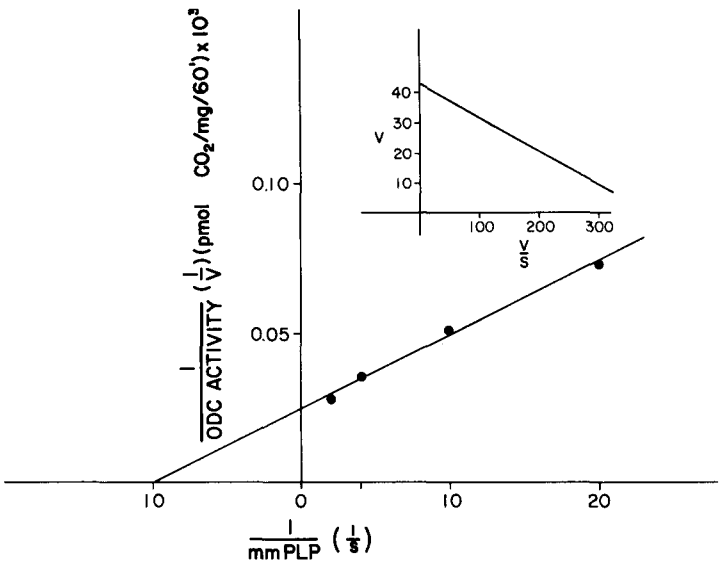

Fig. 4. The effect of varying pyridoxal phosphate concentrations in the presence of a constant ornithine concentration on ornithine decarboxylase activity. Experiments were performed at 30 μM ornithine. Pyridoxal phosphate concentrations from 0.025 to 0.5 mM were used.

$8 \cdot 10^3$ pmol CO_2/mg/h) for the reaction but the K_m for ornithine returns to normal. When the level of pyridoxal phosphate is increased to 3 mM, much of the noncompetive aspect of the inhibition is lost (Fig. 5B). The K_I values for putrescine are 147 μM in the presence of 0.25 mM pyridoxal phosphate and 978 μM in the presence of 3 mM pyridoxal phosphate. These effects can be explained in terms of a model for the reaction (see below).

The effect of putrescine analogs and other related compounds on ornithine decarboxylase activity. Ethylene diamine, 1,3-diaminopropane, 1,5-diaminopentane, spermidine, spermine, and urea were all tested at 1 and 10 mM concentrations. None of the compounds showed any significant effects on the enzyme at the lower concentration, but inhibition was apparent at 10 mM with all compounds (Table III). This inhibition could be overcome by addition of higher levels of pyridoxal phosphate (Table III) and therefore probably represents the effect of polyamine binding to free pyridoxal phosphate.

Spectral changes of the enzyme and pyridoxal phosphate. In Fig. 1, we presented the ultraviolet and visible spectrum of the partially purified ornithine decarboxylase enzyme. Fig. 6 presents the visible spectral data for the holoenzyme (with and without 1 mM ornithine) and free pyridoxal phosphate (with and without 1 mM ornithine); all spectra are at pH 7.

The formation of a Schiff base complex with ornithine and both free pyridoxal phosphate and enzyme bound pyridoxal phosphate is apparent since the maximum at 325 nm for pyridoxal phosphate is flattened to a shoulder and the peak at 388 nm is shifted to 410 nm. Comparable changes occur on the enzyme, the one difference being the enhanced absorbance at 320—330 nm.

Effect of various nucleotides on ornithine decarboxylase activity. Beck et al.

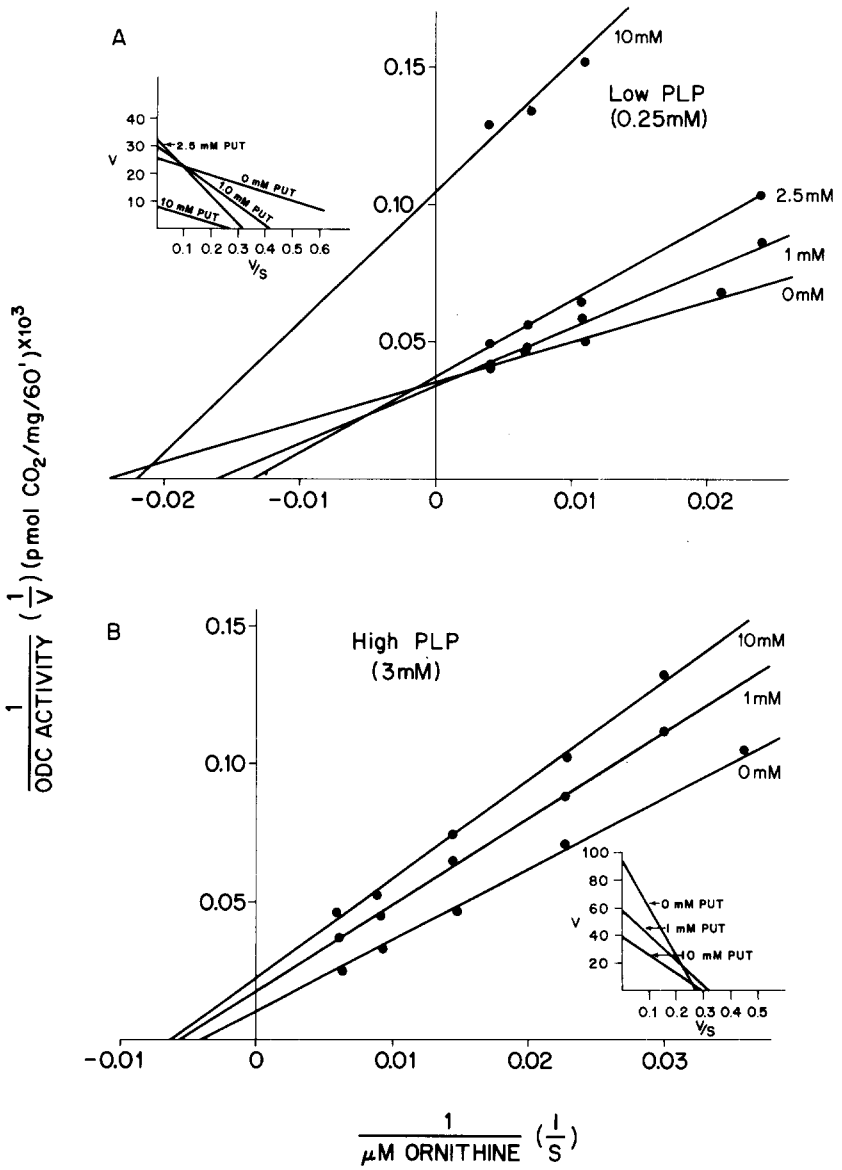

Fig. 5. The effect of putrescine on ornithine decarboxylase activity. Conditions were as follows: (A) 0.025 mM pyridoxal phosphate, and putrescine at 0, 1, 2.5 and 10 mM concentrations (indicated on the line). Ornithine was used at 50—300 μM. (B) 0.3 mM pyridoxal phosphate, and putrescine at 0, 1 and 10 mM. Ornithine was used at 28—200 μM.

[25] showed that dibutyryl cyclic AMP was able to stimulate increases in hepatic ornithine decarboxylase in vivo, suggesting that regulation of this enzyme might be through cyclic AMP. We found that cyclic AMP, deoxy CTP, deoxy ATP, deoxy GTP and ATP had no effect on the mouse liver ornithine decarboxylase.

TABLE III

THE EFFECTS OF HIGH CONCENTRATIONS OF PYRIDOXAL PHOSPHATE ON INHIBITION OF ORNITHINE DECARBOXYLASE BY PUTRESCINE ANALOGS AND SPERMIDINE AND SPERMINE

Polyamine	% Inhibition (0.25 mM pyridoxal phosphate)	% Inhibition (3 mM pyridoxal phosphate)
None *	0	—
1,3 Diaminobutane **	59	18
1,5 Diaminopentane **	57	11
Spermidine **	67	8
Spermine **	71	5

* Reaction mixture contained, pyridoxal phosphate (at prescribed concentration) 0.3 mM [^{14}C]-ornithine · HCl 2.5 mM dithiothreitol and 0.1 M Tricine (pH 7.2).

** Compounds were tested at 10 mM.

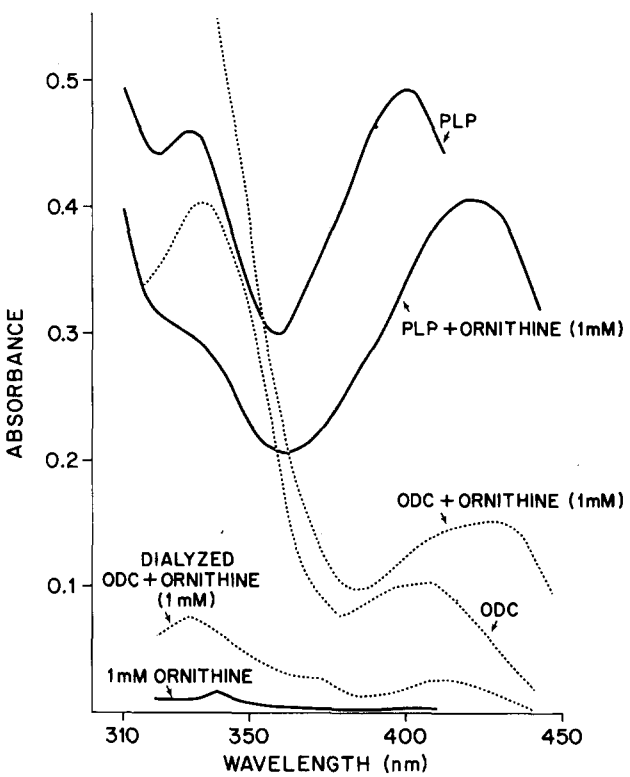

Fig. 6. Visible spectral data for ornithine decarboxylase and pyridoxal phosphate in the absence and presence of ornithine. All spectra were determined at 0.05 M sodium phosphate buffer (pH 7.2). ———, pyridoxal phosphate (PLP) free curves, in the absence and presence of 1 mM ornithine, · · · · · ·, ornithine decarboxylase (ODC) in the absence and presence of 1 mM ornithine. Bottom dotted curve shows the spectrum of the enzyme in the absence of bound pyridoxal phosphate but in the presence of ornithine.

Discussion

Ornithine decarboxylase from mouse liver is similar in many respects to other ornithine decarboxylase enzymes that have been studied [2—5]. Thiol requirements for the mouse liver enzyme were not extensively investigated, although others [2,26] have shown marked differences in rat hepatic ornithine decarboxylase activity, pH optima and molecular weights for the enzyme with various thiols. In this respect it is interesting to note the spectral shift in the 280 nm region to higher wave lengths on addition of dithiothreitol to the enzyme (Fig. 1), this change might indicate a conformational alteration occurring in the enzyme. Since the oxidation state of dithiothreitol affects the spectrum of this compound, such subtle spectral changes as we observed could be also due to changes in oxidation state.

With the partially purified enzyme, the apparent K_m for ornithine was 26 μM. In crude liver homogenates it was 250 μM, probably due to other reactions competing with ornithine decarboxylase for available ornithine.

The spectral data in Figs. 1 and 6 show that pyridoxal phosphate plays an important role in the ornithine decarboxylase reaction. Not only does the binding of ornithine to ornithine decarboxylase enzyme probably take place through Schiff base formation, as indicated by the spectral shifts that occur in the bound pyridoxal phosphate peaks (Fig. 6), but also ornithine may form Schiff base complexes with free pyridoxal phosphate at pH 7 (Fig. 6). Therefore the formation of free pyridoxal phosphate-substrate complexes which are not effective substrates for the enzyme, leads to a form of non-competitive inhibition at high substrate levels.

There are thus two opposing effects of increased pyridoxal phosphate concentrations, at altered substrate level (Fig. 3). A V increase, probably as a result of increased availability of pyridoxal phosphate in the enzyme active site and a K_m increase reflecting a binding of free pyridoxal phosphate to ornithine in a Schiff base complex resulting in a pyridoxal phosphate-substrate complex which is unable to bind effectively to the enzyme.

When studying the effect of putrescine on ornithine decarboxylase kinetics (Fig. 5), we noted that at high putrescine concentrations (10 mM), V is reduced but K_m is returned to normal from an increased value at low (2.5 mM) putrescine. The return of the K_m to normal may be due to release of ornithine from Schiff base complex with pyridoxal phosphate by the preferential binding of the free pyridoxal phosphate to putrescine which is present in excess.

The enzyme is competitively inhibited by low concentrations of putrescine but not by its close analogs. Spermidine and spermine inhibit (non-competitively) only at high concentrations (Table III). That the catalytic site of ornithine decarboxylase is specific for ornithine is indicated by the lack of competitive inhibition by 1,3 diamino propane or 1,5 diaminopentane.

Although Friedman et al. [2] reported some nucleotide inhibition of the rat liver enzyme, we detected no effect of the tested nucleotides including cyclic AMP. (A report by Byus and Russell [27] indicates that cyclic AMP may regulate levels of ornithine decarboxylase in the adrenal medulla and cortex probably via an activation of a protein kinase).

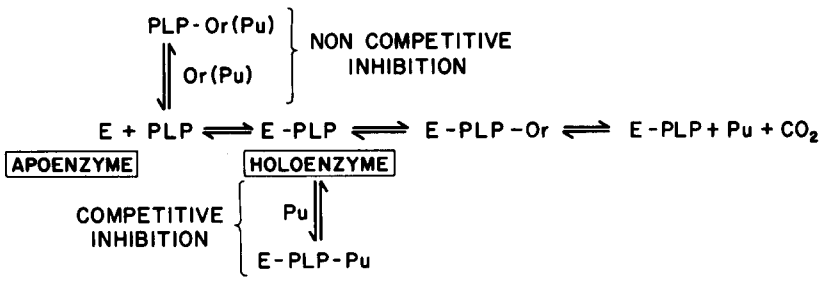

Fig. 7. A possible mechanism for product and cofactor regulation of ornithine decarboxylase. Or = ornithine, Pu = Putrescine, E = ODC apoenzyme, PLP = pyridoxal phosphate. This figure represents the three equilibria involved in the enzyme system, (1) PLP (free) ⇌ PLP (bound) (2) PLP (free) + (Or) (Pu) ⇌ PLP (free) · (Or) (Pu), (3) PLP (enzyme bound) + (Or) (Pu) ⇌ PLP (enzyme bound) · (Or) (Pu). Number 3 gives rise to competitive inhibition with putrescine, increasing K_m but not V, number 2 gives rise to noncompetitive inhibition, decreasing V but not altering K_m.

Proposed regulatory mechanism for ornithine decarboxylase

The following scheme for the regulation of ornithine decarboxylase by metabolites is proposed (Fig. 7) based on the kinetic data reported here. The apoenzyme reacts with free pyridoxal phosphate to form holoenzyme in a reversible fashion (the equilibrium lies somewhat in favor of holoenzyme formation). Addition of moderate levels of ornithine (Or) or putrescine (Pu) causes binding of ornithine or putrescine to the bound pyridoxal phosphate, leading either to products (with ornithine) or competitive inhibition (with putrescine). Addition of excess ornithine or higher levels of putrescine may cause noncompetitive inhibition by removing free pyridoxal phosphate from the pyridoxal phosphate apoenzyme equilibrium, through formation of Schiff bases. With putrescine alone mixed noncompetitive and competitive inhibition arise. Increased levels of pyridoxal phosphate reverse the noncompetitive inhibition by increasing the free pyridoxal phosphate in equilibrium with the enzyme.

It has been shown here that mouse liver ornithine decarboxylase, binding pyridoxal phosphate only moderately well has complex kinetics through the formation of Schiff base complexes with the free cofactor (pyridoxal phosphate) and substrates or inhibitors. The presence of these Schiff bases makes interpretation of kinetic data difficult, except at low substrate/inhibitor concentrations and leads to the possibility that this enzyme is partially regulated through the availability of free pyridoxal phosphate.

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References

1 Russell, D.H. (1970) Ann. N.Y. Acad. Sci. 171, 772—782
2 Friedman, S.J., Halpern, K.V. and Cannellakis, E.S. (1972) Biochim. Biophys. Acta 261, 181—187

3 Janne, J. and Williams-Ashman, H.G. (1971) J. Biol. Chem. 246, 1725—1732
4 Williams-Ashman, H.G., Coppoc, G.L. and Weber, G. (1972) Cancer Res., 32, 1124—1132
5 Hogan, B.L.M. (1971) Biochem. Biophys. Res. Commun. 45, 301—307
6 Panko, W.B. and Kenney, F.T. (1971) Biochem. Biophys. Res. Commun. 43, 346—350
7 Pegg, A.E., Lockwood, D.M. and Williams-Ashman, H.G. (1970) Biochem. J. 117, 17—31
8 Kobayashi, Y., Kupelian, J. and Maudsley, D.V. (1971) Science 172, 379—380
9 Cohen, S., O'Malley, B.W. and Stastny, M. (1970) Science 170, 336—338
10 Williams-Ashman, H.G., Janne, J., Coppoc, G.L., Geroch, M.E. and Schenone, A. (1972) in Advances in Enzyme Regulation, (Weber, G., ed.), Vol. 10, p. 225, Perganon Press, N.Y.
11 Schrock, T.R., Oakman, J.J. and Bucher, N.L.R. (1970) Biochem. Biophys. Acta 204, 564—577
12 Russell, D.H. and Snyder, S.H. (1968) Proc. Natl. Acad. Sci. U.S. 60, 1420—1427
13 Janne, J. and Raina, A. (1968) Acta Chem. Scand. 22, 1349—1351
14 Fausto, N. (1969) Biochim. Biophys. Acta 190, 193—201
15 Raina, A., Janne, J., Hannonen, P. and Holtta, E. (1970) Ann. N.Y. Acad. Sci. 171, 697—708
16 Fausto, N. (1971) Biochem. Biophys. Acta 238, 116—128
17 Snyder, S.H., Kreuz, D.S., Medina, V.J. and Russell, D.H. (1970) Ann. N.Y. Acad. Sci. 171, 749—771
18 Janne, J. and Holtta, K. (1974) Biochem. Biophys. Res. Commun. 61, 449—456
19 Morley, C.G.D. and Kingdon, H.S. (1973) Biochim. Biophys. Acta 308, 260—275
20 Higgins, G.M. and Anderson, R.M. (1931) Arch. Pathol. 12, 186—202
21 Kremzner, L.T. and Pfeiffer, C.C. (1966) Biochem. Pharmacol. 15, 197—200
22 Morley, C.G.D. (1972) Biochem. Biophys. Res. Commun. 49, 1530—1535
23 Morley, C.G.D. (1974) Biochim. Biophys. Acta 362, 480—492
24 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 765—775
25 Beck, W.T., Bellantone, R.A. and Canellakis, E.S. (1972) Biochem. Biophys. Res. Commun. 48, 1649—1655
26 Janne, J. and Williams-Ashman, H.G. (1970) Biochem. J. 119, 595—597
27 Byus, C. and Russell, D.H. (1975) Science 187, 650—652