IN VITRO INACTIVATION OF ORNITHINE DECARBOXYLASE BY A HEAT-LABILE FACTOR FROM RAT VENTRAL PROSTATE

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1. Introduction

Ornithine decarboxylase (ODC, EC 4.1.1.17, the first enzyme in the pathway of polyamine biosynthesis), has one of the shortest in vivo half lives yet reported [1], making it an ideal enzyme for studying enzyme inactivation. Much effort has been applied to the problem of the hormoral stimulation of ornithine decarboxylase activity (see reviews, [2–4]) but the equally important phase of ODC inactivation [5–8] has not been as extensively studied.

The highest specific activity of ODC in mammalian organs [9,10] has been found in gonadotrophin-treated immature rat ovaries 4 h after injection of luteinizing hormone (LH). We also found that the follicular tissue is the ovarian compartment most response to this action of LH [11]. In the present study, we report that the ODC activity in extracts of follicle-rich ovaries is stable in vitro in the absence of pyridoxal phosphate (PLP), in contrast to enzyme preparations from other sources. This provided us with a test system in which we could demonstrate the presence of an ornithine decarboxylase inactivating factor (ODIF) in extracts of young adult prostate glands that can be separated from ODC activity.

2. Materials and methods

2.1. *Hormones and other biochemicals*

Pregnant mare serum gonadotrophin (PMSG, Gestyl) was a product of Organon, Oss, The Netherlands. Ovine luteinizing hormone (NIH-LH-18) was kindly supplied by the National Institute of Arthritis and Metabolic Disease, Bethesda Md. USA [1-^{14}C]DL Ornithine (3.0 mCi/mmol) and [1-^{14}C]S-adenosyl-1-methionine (2.43 mCi/mmol) were purchased from New England Nuclear Corp. Boston Mass. USA. Protamine phosphate was prepared enzymatically [12] from protamine sulfate (Fluka A.G. Buchs, Switzerland).

2.2. *Animals and enzyme extracts*

Female Wistar rats were injected subcutaneously with 10 i.u. PMSG and 52 h later intraperitoneally with 30 μg LH and killed 4 h after the LH injection. The ovaries were dissected free of fat, homogenized in an all-glass Potter-Elvehjem homogenizer (0.005–0.007 in clearance) in 0.25 M sucrose containing 10 mM Tris–HCl, pH 7.5, 5 mM dithiothreitol and 0.1 mM disodium ethylenediaminetetraacetate and centrifuged at 150 000 g_{av} for 30 min. Ventral prostate glands were dissected from 3–4 month old rats, homogenized and centrifuged as described above. The supernatant solutions were subjected to gel filtration on a column (0.7 × 13 cm) of Sephadex G-25 equilibrated with a medium of similar compositions but lacking sucrose (TDE buffer).

Assays for ODC activity were performed at 2 concentrations of enzyme (one double the other) as described previously [10].

3. Results

Ornithine decarboxylase (ODC) activity extracted from the ovaries of rats treated with PMSG and LH

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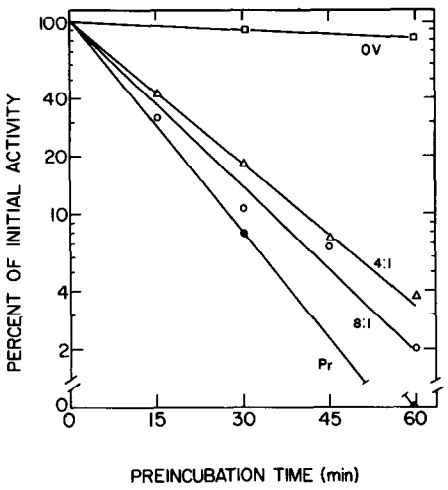

Fig.1. Stability of ODC in extracts from different organs to inactivation at 37°C. High speed supernatant solutions (150 000 *g*) were prepared [10] from either prostate glands of 4 month old rats (●——●) or from ovaries (□——□) of 26 day old gonadotrophin treated rats (v. section 2.2). The supernatant solutions were subjected to gel filtration through a Sephadex G-25 column and preincubated for the indicated periods of time at 37°C before assay of ODC activity as described previously [10]. Mixtures of the two extracts were made at 4:1 (△——△) or 8:1 (○——○) ratio of prostatic to ovarian protein. Initial (100%) activity values were 1.79 nmol $^{14}CO_2$ liberated/h/mg protein and 32.3 nmoles $^{14}CO_2$/h/mg protein for prostatic and ovarian extracts respectively.

has a half life of more than one hour when incubated at 37°C in the absence of its coenzyme pyridoxal phosphate (PLP); an enzyme preparation from ventral prostate gland had a half life of only 8 min (fig.1). The activity of mixtures of the two enzyme preparations (fig.1) showed a half-life of 13 min (4:1 ratio of prostatic to ovarian protein) and 10 min (8:1 ratio) respectively. Inactivation of ovarian ODC by addition of a prostatic extract was not observed (after 30 min) at 0°C or when the prostate extract was boiled before incubation at 37°.

The inactivation of ovarian ODC by a prostatic extract was not inhibited by anti-proteolytic agents such as the Kallikrein inhibitors Trasylol SK/TK/1 (1000 μg/ml, 500 KIU/mg) or Trasylol GOS 746/31 (1000 μg/ml, 5700 KIU/mg) from Bayer A.G. Elberfeld, Germany, benzamidine (8 mg/ml) from Eastman Kodak Co., Rochester, N.Y., USA., tosyl-lysyl-chloromethyl ketone (2 μg/ml) from Sigma Chemical Co., St. Louis, Mo., USA, or phenyl-methyl-sulfonyl chloride (10^{-3} M) and diisopropyl fluoro-phosphate (10^{-3} M) from Calbiochem, A.G., Lucerne, Switzerland.

The specificity of the inactivating factor present in a prostatic extract for ODC compared to other enzymes is shown in table 1. Only ODC showed a loss of activity upon incubation of the prostate extract for 15 min at 37°C whereas the PLP dependent enzyme [13] *S*-adenosyl-methionine decarboxylase (EC. 4.1.1.50, the next enzyme in the pathway of polyamine biosynthesis) showed some increase in activity. Three metabolically unrelated enzymes, phosphoprotein phosphohydrolase (EC 3.1.3.16), glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 6 phospho-gluconate dehydrogenase (EC 1.1.1.47) maintained their activities. ODC was protected against inactivation by 10^{-4} M PLP, and some increase of activity was observed; the other enzymes studied showed no change upon incubation with PLP.

In order to assess quantitatively the ability of a prostatic extract to inactivate ODC, we considered a unit of ODC activity as that activity which catalyzes the release of 1 nmol $^{14}CO_2$/h [10]; we defined a unit of ornithine decarboxylase inactivating factor as that activity which results in a decrease of 1 unit of ODC activity in 1 h preincubation at 37°C. Measurements of ODIF were made using 180 units per ml of ODC as substrate and a 15 min preincubation period. ODIF activity was proportional to protein concentration in the range tested.

When a prostatic extract was subjected to precipitation at pH 4.6 by the method of Ono et al. [14], the precipitate showed a 3-fold increase in the specific activity of ODC accompanied by a halving of the specific activity of ODC inactivating factor (table 2). The supernatant solution showed negligible ODC activity and nearly a doubling in its ODIF specific activity. The ODIF activity in the supernatant solution represented 86% of the initial ODIF activity. An independent fractionation of a second pool of prostate glands resulted in a similar separation of ODC and ODIF activity with an increase of the specific activity of ODC in the pH 4.6 precipitate of 2.4-fold and an increase in the specific activity of ODIF in the supernatant of 2.2-fold.

Table 1
Stability of different prostatic enzyme activities at 37°C in the presence and absence of PLP

Enzyme	% Activity remaining after incubation at 37°C for 15 min	
	Without pyridoxal phosphate	With pyridoxal phosphate
Ornithine decarboxylase	62	129
S-adenosyl-1-methionine decarboxylase	125	109
Phosphoprotein phosphohydrolase	102	102
Glucose 6-phosphate dehydrogenase	96	88
6-Phosphogluconate dehydrogenase	98	99

A high speed supernatant solution (150 000 *g*) was prepared [10] from the prostate glands of 3 month old rats and subjected to gel filtration as described in section 2.2. Analysis of enzymic activities was made before and after incubation for 15 min at 37°C in the presence and in the absence of 10^{-4} M pyridoxal phosphate. Assays for ODC activity [10] and S-adenosyl-1-methionine decarboxylase activity [20] were carried out in the presence of pyridoxal phosphate and based on the release of $^{14}CO_2$ from [1-^{14}C]DL ornithine and [1-^{14}C]*S*-adenosyl-1-methionine. Phosphoprotein phosphohydrolase was assayed [12] by the release of ^{32}P from protamine phosphate (179 counts/pmole phosphate). Glucose 6-phosphate dehydrogenase [21] and 6-phosphogluconate dehydrogenase [21] were assayed spectrophotometrically. The $^{14}CO_2$ radioactivity was detected by liquid scintillation counting [20] and ^{32}P by Cerenkov radiation [22]. The initial (100%) values were: ODC, 2.08 nmol $^{14}CO_2$ liberated/h/mg protein; S-adenosyl-1-methionine decarboxylase, 3.24 nmol $^{14}CO_2$ liberated/h/mg protein; phosphoprotein phosphatase, 1.58 nmol ^{32}P released/mg protein/10 min; glucose 6-phosphate dehydrogenase 0.020/units/mg protein [21]; and 6-phosphogluconate dehydrogenase 0.0158 units/mg protein [21].

4. Discussion

The half life of ODC activity of 8 min in vitro for a prostatic enzyme in the absence of PLP (which is close to that of 12-20 min found for liver ODC in vivo [1] could be due to:

1. Inherent instability of the enzyme.
2. Enzyme modification, or
3. Proteolytic degradation of ODC.

We have shown that a high speed prostatic supernatant which has been freed from low molecular weight constituents by gel filtration through Sephadex G-25 is able to produce a decrease in the activity of an ovarian ODC preparation.

The possibility of inherent instability of prostate ODC is unlikely since a form of ODC which is stable in the absence of PLP is found in gonadotrophin treated ovaries. This enzyme has a K_M for PLP of 0.73 μM cf. [10] while the prostatic ODC (unstable) has a K_M of 2.15 μM, similar enough to suggest, in the absence of any evidence to the contrary, that ODC structure in these two organs is closely related.

ODC activity could be modified by the addition or the removal of certain residues. The possibility that the inactivation of ODC is brought about by addition of low molecular weight groups is rendered unlikely by the fact that the extracts have been subjected to gel filtration for removal of such low molecular weight components. The effectiveness of this procedure is shown by the fact that both ovarian and prostatic extracts show complete dependence on added PLP for activity. The possibility that ODC may be modified and thus inactivated by removal of one or more groups, requires further study.

The third possibility of a proteolytic cleavage of ODC being involved in its inactivation [15] although

Table 2
Separation of the ODC inactivating factor of rat prostate from ODC

Fraction	Total Protein (mg)	ODC Activity (units)	ODIF Activity (units)
Sephadex G-25 filtrate	71.8	333	3487
pH 4.6 pellet	20.5	261	508
pH 4.6 supernatant	37.9	4	3016

A high speed supernatant solution (150 000 *g*) was prepared from the prostate glands of four month old rats as described in section 2.2. The Sephadex (G-25) filtrate was brought to pH 4.6 by gradual addition of 0.5 M acetic acid with constant stirring at 0–4°C. The suspension was centrifuged at 38 000 g_{max} for 5 min at 0°C, and the resulting precipitate was resuspended in 1.5 ml of TDE buffer. The supernatant solution was subjected to gel filtration through a 0.7 × 13 cm column of Sephadex G-25 equilibrated with TDE buffer. ODC inactivating factor activity (ODIF) was determined by incubation, for 15 min at 37°C, of 79–155 μg of protein from a prostatic extract (containing 0.01–1.7 units of ODC activity) in the presence of 290 μg of protein from an extract of ovaries (see 2.2) obtained from gonadotrophin treated rats (containing 9 units of ODC activity) in a total volume of 50 μl. Incubations were terminated by addition of cold buffer containing pyridoxal phosphate, ornithine and DTT, allowing direct determination of the remaining ODC activity as described [10]. Assays for both activities were performed in duplicate. Activity units are defined in section 3.

attractive, has not been proven. However, the recent report of Hölttä [16] that the activity of rat liver ODC after treatment which cycloheximide decreases in parallel with the decrease in immunoreactive enzyme protein suggests a degradation process is involved.

We have not yet found an antiproteolytic agent that prevents the inactivation of ODC, although a variety of agents were tested at or near the maximal concentration which does not interfere with the ODC assay. If, indeed, a proteolytic enzyme is involved in the inactivation of ODC, it is probably not one of the 'group specific proteases' described by Katunuma [15]. The evidence for this is: (a) we do not find a detectable in vitro inactivation of *S*-adenosyl-1-methionine decarboxylase, another PLP dependent enzyme [13], which has a half life in vivo of approximately 60 min [17] and (b) we were unable to suppress the inactivation of ODC by addition of diisopropyl fluorophosphate which is effective against the proteases described by Katunuma as acting specifically against PLP apoenzymes [15].

Pegg and Williams-Ashman [18] and Jänne and Williams-Ashman [9] have reported a protecting effect of PLP on preparations of rat ventral prostate ODC at low temperatures. In a study of the stability of another PLP dependent enzyme, glutamic decarboxylase from *E. coli*, Shukuya and Schwert [19] showed that PLP has a stabilizing effect at 25°C similar to the effect shown on ODC in table 1. Whether the PLP protection of rat prostatic ODC shown here is mediated by a direct effect of the cofactor on the enzyme or by PLP binding to ODIF remains an open question.

The possibility that the ODC inactivating factor reported here is itself under hormonal regulation (which may also be a factor in the variation in the rate of inactivation of ODC in crude prostatic extracts, cf. table 1 and fig.1) is presently being investigated.

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