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Purification of Ornithine Decarboxylase from Kidneys of Androgen-Treated Mice†

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ABSTRACT: Ornithine decarboxylase has been purified to homogeneity from kidneys of androgen-treated mice. Such kidneys have an enzyme content 2 orders of magnitude greater than that of other mammalian tissues such as induced rat liver, and only a 10350-fold purification was needed for purification. The enzyme preparation gave a single band on isoelectric focusing and on polyacrylamide gel electrophoresis under native and denaturing conditions. These bands corresponded to the enzyme activity and to the migration of ornithine decarboxylase labeled by reaction with α-(difluoromethyl)[5-^{14}C]ornithine, a specific inhibitor. The enzyme has a M_r of about 100000 and is a dimer of subunit M_r 53000. The K_m for L-ornithine was 75 μM and for pyridoxal phosphate, 0.3 μM. The preparation had a specific activity of 50 μmol of CO_2 produced min^{-1} mg^{-1} and bound a stoichiometric amount of the irreversible inhibitor, α-(difluoromethyl)ornithine (one molecule per subunit). The purified enzyme was unstable even in the presence of 2.5 mM dithiothreitol and 40 μM pyridoxal phosphate unless 0.02% Brij 35 was added. In the presence of this detergent, the enzyme could be stored with little loss of activity.

There has been considerable interest in the enzyme ornithine decarboxylase (EC 4.1.1.17) for a number of reasons [reviewed by Morris & Fillingame (1974), Jänne et al. (1978), Canellakis et al. (1979), McCann (1980), Russell (1980), and Pegg & Williams-Ashman (1981)]. In mammalian cells, this enzyme provides the only source of putrescine, a precursor of the polyamines. Its activity increases very rapidly in response to a wide variety of trophic stimuli, and it appears to have the most rapid rate of protein synthesis and degradation among mammalian enzymes. Its activity may also be regulated by the level of a macromolecular inhibitor (Canellakis et al., 1978) and a variety of posttranslational modifications (Mitchell, 1981; Russell, 1981; Atmar & Kuehn, 1981). The enzyme is the target for potentially useful chemotherapeutic agents that block polyamine production (Sjoerdsma, 1981; Heby & Jänne, 1981). Despite the intense interest in ornithine decarboxylase, the mammalian enzyme has proved difficult to purify. Homogeneous preparations of ornithine decarboxylase have been obtained in milligram quantities from bacteria (Applebaum et al., 1975, 1977; Guirard & Snell, 1980) and from yeast and slime mold (Tyagi et al., 1981; Atmar & Kuehn, 1981; Mitchell et al., 1978) but not from mammalian tissues. Highly purified preparations have been reported for the enzyme from rat prostate (Jänne & Williams-Ashman, 1971), rat liver (Ono et al., 1972; Obenrader & Prouty, 1977; Pegg & McGill, 1979; Kitani & Fujisawa, 1981), calf liver (Haddox & Russell, 1981), mouse fibroblasts (Weiss et al., 1981), and mouse kidney (Persson, 1981a,b), but convincing evidence that these preparations are homogeneous was not given. In fact, numerous preparations of rat liver ornithine decarboxylase have been published, each describing the purification of a material giving a single band on polyacrylamide gel electrophoresis but increasing the specific activity of the purified material (Friedman et al., 1972; Ono et al., 1972; Hölttä, 1975; Obenrader & Prouty, 1977; Pegg & McGill, 1979; Kitani & Fujisawa, 1981). A recent preparation notes that the material was not homogeneous when tested by isoelectric focusing, although the specific activity of 1 μmol of CO_2 min^{-1} mg^{-1} was greater than those previously published (Kitani

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& Fujisawa, 1981). This finding is in agreement with our prediction that rat liver ornithine decarboxylase should have a specific activity of 20–25 μmol min^{-1} mg^{-1} when homogeneous (Pritchard et al., 1981; Pegg et al., 1981). This prediction was based on the titration of the enzyme with labeled α-(difluoromethyl)ornithine (DFMO),[1] an enzyme-activated irreversible inhibitor (Metcalf et al., 1978). Assuming the stoichiometric binding of this inhibitor for every molecule of enzyme inactivated, we calculated that even when fully induced, rat liver contains less than 0.1 ng of enzyme/g of tissue (Pritchard et al., 1981). Therefore, more than 2000 rats would be required to obtain 1 mg of enzyme if the isolation procedure gave a 50% yield. Very recently, Kameji et al. (1981) have reported without full experimental details the purification of rat liver ornithine decarboxylase to almost this predicted specific activity. They obtained only 25 μg of enzyme from 150 rat livers. The very small amount of ornithine decarboxylase present in this and most other mammalian sources probably accounts for the majority of the difficulty in purifying this enzyme. Other problems may relate to the existence of multiple forms of the enzyme (Clark & Fuller, 1976; Obenrader & Prouty, 1977; Canellakis et al., 1978; Lau & Slotkin, 1979; Haddox & Russell, 1981; Richards et al., 1981) and the extreme lability of the enzyme when partially purified and in dilute solution. Another difficulty has been the absence of convincing criteria for purity since as described above the presence of a single band on polyacrylamide gel electrophoresis has not proved satisfactory.

In the present paper we describe the purification of ornithine decarboxylase and some properties of the enzyme. Kidneys from androgen-treated mice were used as a starting material. Such treatment greatly enhances ornithine decarboxylase activity in the mouse kidney (Henningsson & Rosengren, 1975; Pegg & McGill, 1979), providing a starting material having a specific activity more than 100 times greater than hepatotoxin-induced rat liver (Ono et al., 1972; Pegg & McGill, 1979) and more than 400 times greater than induced calf liver (Haddox & Russell, 1981). The lability of the enzyme was prevented by addition of detergent (Pritchard et al., 1981) and the use of a rapid procedure in which the enzyme was maintained in the presence of dithiothreitol (Jänne & Williams-Ashman, 1971). Finally, the homogeneity of the preparation was monitored by titration with DFMO (Pritchard et al., 1981; Pegg et al., 1981) and by the coincidence of the enzyme with DFMO-labeled protein on isoelectric focusing, denaturing and native gels. An abstract describing parts of this work has been published (Seely et al., 1982).

Experimental Procedures

Assay of Ornithine Decarboxylase. Ornithine decarboxylase activity was determined by measuring the release of $^{14}CO_2$ from L-[1-^{14}C]ornithine. The assay mixture contained 0.4 mM L-ornithine, 0.125 μCi of L-[1-^{14}C]ornithine (New England Nuclear, Boston, MA) (57 mCi/mmol), 0.04 mM pyridoxal 5′-phosphate, 1.25 mM dithiothreitol, 50 mM Tris-HCl, pH 7.5, and enzyme in a total volume of 0.25 mL. The reaction was carried out in test tubes closed with rubber stoppers carrying polypropylene wells containing 0.2 mL of 1 M hyamine hydroxide. The assay was started by the addition of enzyme, and the tubes were incubated at 37 °C for 15 min in a shaking water bath. The reaction was stopped by the injection of 0.3 mL of 5 M sulfuric acid through the rubber cap. After a further 30 min, the well and contents were placed in 5 mL of a toluene-based scintillation fluid and assayed for radioactivity. Blank tubes were set up in which sulfuric acid was added before the enzyme. One unit of enzyme activity was defined as the amount releasing 1 nmol of CO_2/min.

Protein Determination. Protein was determined by the method of Bradford (1976) using bovine liver aldolase as a protein standard. We chose not to use bovine serum albumin as a standard for protein determination since it binds Coomassie dye to a greater extent than most proteins (Read & Northcote, 1981), leading to an erroneously low value for protein concentration. Bovine liver aldolase bound approximately half as much dye reagent as bovine serum albumin, giving us a specific activity half that obtained with bovine serum albumin as a standard.

Binding of [^{14}C]DFMO to Ornithine Decarboxylase. In experiments where the amount of [^{14}C]DFMO binding to ornithine decarboxylase was determined, the enzyme was incubated with 0.04 mM pyridoxal 5′-phosphate and 5 μM [^{14}C]DFMO (Amersham/Searle, Arlington Heights, IL; 60 mCi/mmol) for 60 min at 37 °C in a shaking water bath. At the end of the incubation, the protein was precipitated by the addition of 1 M perchloric acid. The protein pellet was washed twice with 1 M perchloric acid, in chloroform–ethanol–ether (1:2:1), and once in ether. The protein pellet was then allowed to dry and was dissolved in 0.1 M sodium hydroxide at 100 °C and counted in ACS II scintillation fluid. When highly purified preparations of ornithine decarboxylase were used, 10 mg of bovine serum albumin was added prior to the 1 M perchloric acid to serve as a carrier. In experiments where the labeled protein was analyzed on polyacrylamide gels and isoelectric focusing plates the sample was dialyzed exhaustively against 25 mM Tris-HCl containing 2.5 mM dithiothreitol, 0.1 mM EDTA, and 0.02% Brij 35 to ensure complete removal of unbound [^{14}C]DFMO.

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis of purified native ornithine decarboxylase was carried out by using 7.5% acrylamide cylindrical gels (0.6 × 11 cm) with 3% stacking gel (0.6 × 1.5 cm) in 25 mM Tris-HCl, pH 7.5, containing 5 mM dithiothreitol and 0.02% Brij 35 at 2 mA/gel for 7 h at 4 °C. Gels were then either stained with Coomassie brilliant blue or cut into 2.2-mm slices for determination of enzyme activity. Enzyme was eluted from the slices by shaking overnight in 0.5 mL of 25 mM Tris-HCl, pH 7.5, containing 5 mM dithiothreitol, 0.02% Brij 35, and 0.1 mM pyridoxal 5′-phosphate at 4 °C. Enzyme activity was determined by adding 1 μCi of L-[^{14}C]ornithine (57 mCi/mmol) and incubating at 37 °C in a shaking water bath for 90 min.

Polyacrylamide Gel Electrophoresis under Denaturing Conditions Carried Out in the Presence of NaDodSO$_4$. The enzyme or [^{14}C]DFMO-labeled enzyme was heated to 100 °C for 2 min in the presence of 2.5% NaDodSO$_4$, 5% β-mercaptoethanol, 5 mM dithiothreitol, 20% glycerol, and 0.001% bromophenol blue. The denatured material was subjected to electrophoresis as described by Laemmli (1970) on either 10% or 7.5% acrylamide gels with a 3.5% stacking gel at 3.5 mA/gel for 7 h. Phosphorylase *b* (94 000), bovine serum albumin (68 000), ovalbumin (43 000), and carbonic anhydrase (30 000) were used as standards for molecular weight determination. The gels were then either stained with Coomassie brilliant blue or sliced into 2.2-mm slices for determination of radioactivity. The radiolabeled protein was eluted from the gel by shaking overnight in 1.0 mL of protosol. The samples were then placed in 10 mL of toluene-based scintillation fluid

[1] Abbreviations: DFMO, α-(difluoromethyl)ornithine; NaDodSO$_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

Table I: Purification of Ornithine Decarboxylase from Mouse Kidney[a]

fraction	total protein (mg)	total units ($\times 10^{-3}$)	sp act. (units/mg)	x-fold purification	yield (%)	[^{14}C]DFMO bound (fmol/unit)	estimated purity (%)
supernatant	4550	21.8	4.8	1	100	423	0.01
dialyzed $(NH_4)_2SO_4$ precipitate	1770	15.5	9.1	2	71	435	0.02
DEAE-cellulose eluate	246	12.2	43.5	9	57	492	0.1
pyridoxamine 5′-phosphate Affi-Gel eluate	0.61	7.8	12 800	2690	36	ND	29
Ultrogel AcA 34 eluate	0.088	4.4	49 667	10350	20	459	111

[a] ND = not determined. Purity was estimated by assuming that 1 fmol of enzyme as determined by DFMO binding was equal to 53 pg of protein.

and counted for radioactivity. In later experiments, the gels were first stained with Coomassie, and the protein band was cut out and counted for radioactivity.

Isoelectric Focusing. Isoelectric focusing was carried out on LKB 1804-101 polyacrylamide isoelectric focusing plates (pH 3.5–9.5). Plates were run at 870 V and 20 mA for 200 min at 10 °C. After the run, the plates were stained with Coomassie brilliant blue or sliced into 2.2-mm slices and counted for radioactivity as described for $NaDodSO_4$–polyacrylamide gels. When native enzyme was run on isoelectric focusing plates, the enzyme was eluted by incubating gel slices in 0.5 mL of 500 mM Tris-HCl, pH 7.5, containing 5 mM dithiothreitol, 0.02% Brij 35, and 0.1 mM pyridoxal 5′-phosphate overnight at 4 °C. Enzyme activity was determined by adding 1 μCi of L-[1-^{14}C]ornithine to each slice and incubating at 37 °C as described above.

Purification of Mouse Kidney Ornithine Decarboxylase. Approximately 100 male Crl:CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA) were treated with testosterone propionate (100 mg/kg) by subcutaneous injection of a solution of 4 mg/mL in sesame seed oil. After 3 days the mice were killed by cervical dislocation and the kidneys homogenized in 2 volumes of buffer A (25 mM Tris-HCl, pH 7.5, containing 2.5 mM dithiothreitol and 0.1 mM EDTA). The homogenate was centrifuged at 100000*g* for 50 min. This and subsequent steps were carried out at 0–4 °C.

Ammonium Sulfate Fractionation. For each 100 mL of supernatant 16.5 g of solid "enzyme-grade" ammonium sulfate was added with stirring. The precipitate was discarded and additional ammonium sulfate (13.5 g/100 mL) was added to the supernatant. The resulting precipitate was dissolved in buffer A and dialyzed overnight against 50 volumes of buffer A.

DE-52 Chromatography. The dialyzed fraction was loaded onto a DE-52 column (2.5 × 25 cm) that had previously been equilibrated with buffer A. The column was washed with approximately 150 mL of buffer A, followed by 150 mL of buffer A containing 0.08 M sodium chloride. Ornithine decarboxylase activity was eluted by using a linear gradient (300 mL) of 0.08–0.3 M sodium chloride in buffer A. The active fractions were pooled, concentrated 10–20-fold in an Amicon pressure cell with a Diaflo PM10 membrane, and dialyzed overnight against 50 volumes of buffer B (25 mM Tris-HCl, pH 7.5, containing 2.5 mM dithiothreitol, 0.1 mM EDTA, and 0.02% Brij 35).

Affinity Chromatography on Pyridoxamine 5′-Phosphate–Agarose. An affinity absorbent was prepared by the reaction of pyridoxamine 5′-phosphate with Affi-Gel 10 (Bio-Rad Laboratories, Richmond, VA) as described by the supplier. The affinity matrix was used essentially as described by Boucek et al. (1978). Concentrated enzyme from the DE-52 fractions was applied to a column (1.6 cm × 8.0 cm) of the agarose–pyridoxamine 5′-phosphate at a flow rate of 1.5 mL/h. The column was washed with 2 column volumes of buffer B at 2.0 mL/h, followed by 4 column volumes of buffer B at 100–150 mL/h. The enzyme was then eluted with buffer B containing 10 μM pyridoxal 5′-phosphate at a flow rate of 2.0 mL/h. Fractions containing enzyme activity were concentrated by ultrafiltration into a volume of 1.2 mL.

Ultrogel AcA 34 Filtration. The concentrated enzyme from the affinity chromatography step was applied to a column (1.6 cm × 65 cm) of Ultrogel AcA 34 that had been equilibrated with buffer B containing 10 μM pyridoxal 5′-phosphate. Elution was carried out with the same buffer at a flow rate of 10 mL/h, collecting 1.5-mL fractions. Those fractions containing enzyme activity (usually fractions 48–60) were pooled and concentrated by ultrafiltration.

Determination of Kinetic Parameters. The K_m for L-ornithine was determined by using assay conditions identical with those described above except that 0.5 μCi of [^{14}C]-ornithine was used in each assay and the final concentration of L-ornithine was varied from 0.0175 to 5.0 mM. The K_m for pyridoxal 5′-phosphate was determined as described above except that a 100 mM sodium phosphate buffer (pH 7.5) was used instead of Tris-HCl and the final L-ornithine concentration was 0.1 mM. The concentration of pyridoxal 5′-phosphate was varied from 0.05 to 100 μM.

Results

Purification of Ornithine Decarboxylase. A summary of the purification of ornithine decarboxylase from mouse kidney is shown in Table I. Approximately 100 μg of enzyme was obtained from 100 mice with a 20% yield. At each stage of the purification the amount of [^{14}C]DFMO that was bound when the enzyme was completely inactivated by this drug was determined. This value was about 423 fmol/unit in the crude extracts and changed insignificantly during the purification (Table I). The M_r of mouse ornithine decarboxylase subunits was 53 000 (see Figures 2 and 4), and on the assumption that only one molecule of drug is needed to inactivate the enzyme the homogeneous enzyme would be expected to have a specific activity of 44 700 units/mg. The degree of purity is calculated in Table I, and the final preparation is in good agreement with this prediction. The slightly higher value actually obtained may relate to the choice of standard protein for the protein assay since the extent of dye binding can vary substantially with different proteins (Read & Northcote, 1981).

Purity of Preparation. The final preparation of ornithine decarboxylase gave a single band on polyacrylamide gel electrophoresis under nondenaturing conditions and in the presence of $NaDodSO_4$ and on isoelectric focusing. These bands coincided with the protein labeled by DFMO and (except for the electrophoresis under denaturing conditions where activity could not be measured) with the enzyme activity that

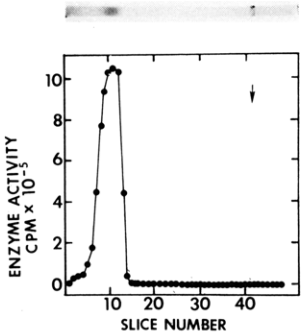

FIGURE 1: Polyacrylamide gel electrophoresis of purified enzyme. Five micrograms of purified enzyme was subjected to electrophoresis on 7.5% gels. The gel was sliced into 2.2-mm slices and the enzyme activity determined in each slice as described under Experimental Procedures. Activity is expressed as cpm of CO_2 released per slice per 90 min (●). The arrow indicates the position of the tracking dye.

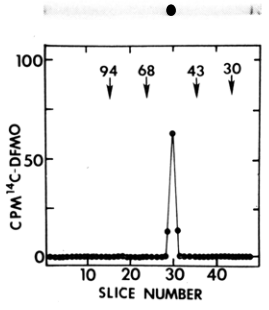

FIGURE 2: $NaDodSO_4$–polyacrylamide gel electrophoresis of purified [^{14}C]DFMO-labeled enzyme. The purified [^{14}C]DFMO-labeled enzyme was denatured as described under Experimental Procedures, and 2 μg was subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis in 10% polyacrylamide gels. The gel was sliced into 2.2-mm slices and the radioactivity in each slice determined as described under Experimental Procedures. The standard protein markers (arrows) used were phosphorylase *b* (94 000), bovine serum albumin (68 000), ovalbumin (43 000), and carbonic anhydrase (30 000).

could be eluted from the gel (Figures 1–3).

Figure 1 shows results when about 5 μg of the purified material was subjected to polyacrylamide gel electrophoresis under nondenaturing conditions. Only one protein band was detected, and this band comigrated with ornithine decarboxylase activity as determined on gels run in parallel.

Figure 2 shows the analysis of the denatured, purified protein on polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$. A single band was obtained that corresponded to an M_r of 53 000. This protein comigrated with the [^{14}C]-DFMO-labeled enzyme in gels run in parallel (Figure 2). In addition, when the Coomassie-stained protein band was cut out and counted, it was found to contain the [^{14}C]DFMO-labeled enzyme. No radioactivity was detected in regions of the gel outside the protein band.

The purified protein (about 2 μg) was subjected to thin-layer isoelectric focusing over a range of 3.5–9.5. The protein and enzyme activity focused at about pH 4.8, and the [^{14}C]-DFMO-labeled enzyme was also present at this place (Figure 3). A minor band that had activity was also seen that comigrated with a second smaller peak of radioactivity. This band was most likely due to a small amount of material that did not penetrate the gel and hence failed to migrate from its point of origin.

Properties of Purified Enzyme. From the elution position of the native enzyme on gel filtration on Ultrogel AcA 34, the M_r of the enzyme was approximately 100 000 (results not shown). The enzyme, therefore, appears to be a dimer containing two 53 000 molecular weight subunits. This is slightly

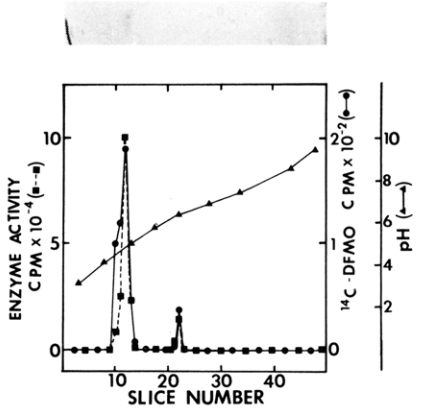

FIGURE 3: Isoelectric focusing of purified enzyme. Two micrograms of the purified [^{14}C]DFMO-labeled enzyme was subjected to thin-layer polyacrylamide isoelectric focusing (pH range 3.5–9.5). Native enzyme (0.2 μg) was focused in an identical run. [^{14}C]DFMO radioactive protein (●) and enzyme activity (■) were determined as described under Experimental Procedures. The pH gradient (▲) at the end of the run was determined by a surface pH electrode.

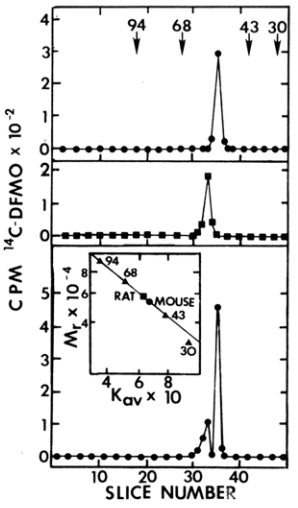

FIGURE 4: $NaDodSO_4$–polyacrylamide gel electrophoresis of [^{14}C]-DFMO-labeled mouse and rat enzymes. Ornithine decarboxylase from thioacetamide-treated rat liver was prepared as described by Pritchard et al. (1981). The mouse kidney enzyme and rat liver enzyme were labeled with [^{14}C]DFMO as described under Experimental Procedures. $NaDodSO_4$–polyacrylamide gel electrophoresis was carried out on 7.5% polyacrylamide gels. The gels were cut into 2.2-mm slices and the radioactivity in each slice was determined as described under Experimental Procedures. (Top panel) $NaDodSO_4$–polyacrylamide gel electrophoresis of [^{14}C]DFMO-labeled mouse kidney enzyme (●). (Middle panel) $NaDodSO_4$–polyacrylamide gel electrophoresis of [^{14}C]DFMO-labeled rat liver enzyme (■). (Bottom panel) $NaDodSO_4$–polyacrylamide gel electrophoresis of combined [^{14}C]DFMO-labeled mouse kidney and rat liver enzymes (●). Molecular weight markers were the same as those in Figure 2.

lower than the reported value for the subunit molecular weight of the enzyme from rat liver (Obenrader & Prouty, 1977; Pritchard et al., 1981). This suggests a significant difference between the enzymes from these two species and is not due to minor variations in protocols between different laboratories. As shown in Figure 4, when the [^{14}C]DFMO-labeled mouse kidney enzyme was mixed with the [^{14}C]DFMO-labeled rat liver enzyme and subjected to polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$, the enzymes could be resolved into two peaks of M_r 53 000, corresponding to the mouse enzyme, and 55 000, corresponding to the rat enzyme. The enzymes also differed in isoelectric point since the rat enzyme focused at pH 4.1 (results not shown), in agreement with the value reported by Ono et al. (1972).

Table II: Preparations of Purified Ornithine Decarboxylase

source	sp act. (μmol of CO_2 released min^{-1} mg^{-1})	subunit M_r	reference
Escherichia coli (biodegradative)	130	2 × 80 000	Applebaum et al. (1975)
Escherichia coli (biosynthetic)	99	2 × 82 000	Applebaum et al. (1977)
Lactobacillus sp. 30a	180	12 × 85 000	Guirard & Snell (1980)
Saccharomyces cerevisiae	0.5	1 × 68 000	Tyagi et al. (1981)
Physarum polycephalum	1.7	2 × 80 000	Mitchell et al. (1978)
regenerating rat liver	0.003	66 000	Friedman et al. (1972)
thioacetamide-treated rat liver	0.19	2 × 55 000	Ono et al. (1972)
thioacetamide-treated rat liver	0.24	2 × 55 000	Obenrader & Prouty (1977), Pegg & McGill (1979), and Pritchard et al. (1981)
thioacetamide-treated rat liver	1.04	ND	Kitani & Fujisawa (1981)
thioacetamide-treated rat liver	19	2 × 50 000	Kameji et al. (1981)
thioacetamide-treated calf liver	0.29	54 000	Haddox & Russell (1981)
mouse 3T3 or SV3T3 cells	2.0	2 × 55 000	Boucek & Lembach (1977) and Weiss et al. (1981)
androgen-treated mouse kidney	50	2 × 53 000	this paper
androgen-treated mouse kidney	12	ND	Persson (1981a,b)

The K_m for L-ornithine for the purified mouse kidney ornithine decarboxylase was found to be 75 μM, which is in reasonable agreement with that found for the enzyme purified from mouse fibroblasts (Boucek & Lembach, 1977; Weiss et al., 1981) but is somewhat lower than that observed for the enzyme from rat prostate (Jänne & Williams-Ashman, 1971), rat liver (Ono et al., 1972; Obenrader & Prouty, 1977; Pegg & McGill, 1979), or calf liver (Haddox & Russell, 1981). The K_m for pyridoxal 5′-phosphate was 0.3 μM, which agrees with that reported for the rat liver (Obenrader & Prouty, 1977) or mouse fibroblast (Weiss et al., 1981) enzyme.

In later stages of purification the enzyme was quite unstable unless 0.02% Brij 35 was present in the preparation. In the presence of Brij, the enzyme could be incubated at 37 °C for at least 30 min with no loss in enzyme activity. The purified enzyme could also be stored for several days at 0–4 °C and for up to 3 weeks at −70 °C with little or no loss in enzyme activity.

Discussion

In the present paper, the purification of homogeneous ornithine decarboxylase from mouse kidney is described. The enzyme was found to be homogeneous by the following criteria: (1) The final specific activity obtained was equal to the predicted specific activity for a purified enzyme on the basis of the amount of [^{14}C]DFMO binding per unit of enzyme activity. (2) A single protein band, which comigrated with the enzyme activity, was obtained when the purified enzyme was subjected to polyacrylamide gel electrophoresis under nondenaturing conditions. (3) A single protein band, which comigrated with the [^{14}C]DFMO-labeled enzyme, was obtained when the purified enzyme was subjected to polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$. (4) A single protein band, which comigrated with the [^{14}C]-DFMO-labeled enzyme, was obtained when the purified enzyme was subjected to thin-layer isoelectric focusing over the pH range 3.5–9.5. It is perhaps slightly unexpected that the enzyme with DFMO attached is not sufficiently different in charge to separate from the native enzyme on isoelectric focusing, but it should be noted that the gels as used for this step had a wide pH range and a difference of 0.1 unit would not be resolved with the slices taken.

Radiolabeled DFMO is an invaluable tool in establishing the purity of ornithine decarboxylase since it attaches covalently to the enzyme. It is, therefore, possible to determine the amount of the enzyme under conditions where enzyme activity is absent such as polyacrylamide gel electrophoresis under denaturing conditions. Many investigators obtained a single band for ornithine decarboxylase from rat liver under such conditions but could not determine unequivocally that the protein was only ornithine decarboxylase (Friedman et al., 1972; Ono et al., 1972; Obenrader & Prouty, 1977; Pegg & McGill, 1979; Kitani & Fujisawa, 1981). Kitani & Fujisawa (1981) and Kameji et al. (1981) have shown that these preparations are not homogeneous as predicted by our studies with labeling by [^{14}C]DFMO (Pritchard et al., 1981).

Our preparation of ornithine decarboxylase from mouse kidney has about a 25-fold greater specific activity than that obtained from cultured mouse fibroblasts by a similar preparative method (Weiss et al., 1981). Part of this difference may be accounted for by the instability of the fibroblast enzyme in very dilute solutions and to differences in the method of measuring protein. However, the mouse kidney has higher activity than cultured cells even when the cells are taken at the peak after serum stimulation (Bethell & Pegg, 1979; Weiss et al., 1981) and provides larger quantities of a better starting material for large-scale purification. While our experiments were in progress, Persson (1981a,b) has also described a purification of mouse kidney ornithine decarboxylase to a somewhat lower specific activity than given here but which is higher than that from any other eukaryotic source.

As shown in Table II, the specific activity of the purified mouse ornithine decarboxylase is comparable to that of bacterial ornithine decarboxylase preparations. It is much greater than that for ornithine decarboxylase obtained from the lower eukaryotes, *Saccharomyces cerevisiae* and *Physarum polycephalum*. The reason for this is unclear but could relate to the presence of inactive enzyme in these preparations since it is known that activity is lost in these microorganisms in response to a posttranslational modification (Mitchell, 1981; Mitchell et al., 1978; Tyagi et al., 1981). Very recently, Kameji et al. (1981) have purified rat liver ornithine decarboxylase to the specific activity predicted by our experiments on the binding of [^{14}C]DFMO to this enzyme (Pritchard et al., 1981), which is about half of that predicted for the mouse enzyme. However, the very small amount of ornithine decarboxylase present in this tissue renders it extremely difficult to obtain much material from this source, and it appears that the mouse kidney is a much better source of enzyme for structural studies and for the preparation of antibodies. These studies are in progress, but it should be noted that there may be significant species variations in ornithine decarboxylase. The rat and mouse enzymes differ in size and isoelectric point as well as in turnover number as predicted by [^{14}C]DFMO binding. As can be seen from Table II, there are significant differences in subunit size and composition among the prep-

arations of ornithine decarboxylase so far described, although the preparations from rodent cells all appear to be dimers with subunit M_r in the range of 50 000–60 000. It is conceivable that interspecies variations are sufficiently great that the preparation of calf liver ornithine decarboxylase described recently by Haddox & Russell (1981) is actually homogeneous even though it had a specific activity 170 times less than that of our preparation. However, no convincing evidence for such homogeneity was obtained by Haddox & Russell (1981), and the small amount of enzyme present in this tissue renders purification and characterization very difficult. The 100000*g* supernatant fraction from androgen-treated mouse kidneys has a specific activity at least 400 times that obtained from thioacetamide-treated calf liver. As shown here, the mouse kidney enzyme can be purified to homogeneity in a relatively simple four-step procedure.

No evidence for multiple forms of the enzyme was obtained in the present work, although it is possible that other forms are lost during the preparation. Our finding that a nonionic detergent, Brij 35, could be used to stabilize the mouse enzyme and the rat liver enzyme (Pritchard et al., 1981) is in agreement with the report of Kitani & Fujisawa (1981) that other detergents can be used to preserve activity in preparations of the rat liver enzyme. The presence of Brij 35 was essential for maintenance of enzyme activity when the purified enzyme was stored in a dilute form.

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