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Measurement of the number of ornithine decarboxylase molecules in rat and mouse tissues under various physiological conditions by binding of radiolabelled α-difluoromethylornithine

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The binding of α-difluoromethylornithine, an irreversible inhibitor, to ornithine decarboxylase was used to investigate the amount of enzyme present in rat liver under various conditions and in mouse kidney after treatment with androgens. Maximal binding of the drug occurred on incubation of the tissue extract for 60 min with 3 μM-difluoromethyl[5-^{14}C]ornithine in the presence of pyridoxal phosphate. Under these conditions, only one protein became labelled, and this corresponded to ornithine decarboxylase, having M_r about 100000 and subunit M_r about 55000. Treatment of rats with thioacetamide or carbon tetrachloride or by partial hepatectomy produced substantial increases in ornithine decarboxylase activity and parallel increases in the amount of enzyme protein as determined by the extent of binding of difluoromethyl[5-^{14}C]ornithine. Similarly, treatment with cycloheximide or 1,3-diaminopropane greatly decreased both the enzyme activity and the amount of difluoromethyl-[5-^{14}C]ornithine bound to protein. In all cases, the ratio of drug bound to activity was 26 fmol/unit, where 1 unit corresponds to 1 nmol of substrate decarboxylated in 30 min. These results indicate that even after maximal induction of the enzyme in rat liver there is only about 1 ng of enzyme present per mg of protein. When mice were treated with androgens there was a substantial increase in renal ornithine decarboxylase activity, the magnitude of which depended on the strain. There was an excellent correspondence between the amount of activity present and the capacity to bind labelled α-difluoromethylornithine in the mouse kidney extracts, but in this case the ratio of drug bound to activity was 14 fmol/unit, suggesting that the mouse enzyme has a higher catalytic-centre activity. After androgen induction, the mouse kidney extracts contain about 170 ng of enzyme/mg of protein. These results indicate that titration with α-difluoromethylornithine provides a valuable method by which to quantify the amount of active ornithine decarboxylase present in mammalian tissues, and that the androgen-treated mouse kidney is a much better source for purification of the enzyme than is rat liver.

L-Ornithine decarboxylase (EC 4.1.1.17) is the first enzyme in the mammalian polyamine-biosynthesis pathway (Tabor & Tabor, 1976; Jänne *et al.*, 1978; Pegg & Williams-Ashman, 1981). This enzyme has been studied extensively in many laboratories, who have shown very rapid and manyfold changes in activity in response to hormones, drugs, toxins and proliferative stimuli (reviewed by Morris & Fillingame, 1974; Jänne *et al.*, 1978; Russell & Durie, 1978; Russell, 1980; Bachrach, 1980). Ornithine decarboxylase activity declines very rapidly on exposure to inhibitors of protein synthesis, suggesting that the protein turns over very rapidly, although alternative explanations of this phenomenon have been advanced (Canellakis *et al.*, 1979).

Most of the studies of changes in ornithine decarboxylase levels have been limited to measurements of enzyme activity, although some attempts have been made to quantify the amount of enzyme actually present by the use of immunological techniques (Hölttä, 1975; Canellakis & Theoharides, 1976; Theoharides & Canellakis, 1976; Oben-

Abbreviation used: DFMO, α-difluoromethylornithine.

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rader & Prouty, 1977; Kallio *et al.*, 1977, 1979; Weiss *et al.*, 1981). Theoretically, such work should provide an unequivocal answer to the question of whether changes in ornithine decarboxylase activity are brought about by changes in the amount of enzyme protein, but, for a variety of reasons, none of these studies is entirely conclusive. As discussed in several reviews (Canellakis *et al.*, 1978, 1979; Pegg & Williams-Ashman, 1981), the antisera produced to mammalian ornithine decarboxylase have not been fully characterized and have a rather low titre. In some cases, antisera were raised to an impure enzyme preparation and then purified by exposure to extracts from tissues not induced for ornithine decarboxylase. It is quite possible that antibodies for other inducible proteins might still be present in such extracts (Canellakis *et al.*, 1978, 1979). These difficulties are due to the very small amount of enzyme present in the cell (Pritchard *et al.*, 1981) and its extreme lability, which has rendered the purification of sufficient enzyme for antibody production extremely difficult. Furthermore, the amount of enzyme present in mammalian cells is so small that only in few cases (in which very large amounts of radioactivity were administered under conditions where enzymic activity was high) could enzyme amounts be assessed by immunochemical precipitation of the labelled protein (Theoharides & Canellakis, 1976; Canellakis & Theoharides, 1976; Obenrader & Prouty, 1977). Most of the measurements of ornithine decarboxylase protein were therefore performed by the titration of activity with antiserum (Hölttä, 1975; Obenrader & Prouty, 1977; Kallio *et al.*, 1977, 1979). This method is time-consuming, difficult to perform with precision and gave contradictory results when the titration was done by different methods in the same laboratory (Kallio *et al.*, 1977, 1979). Therefore, independent methods for assessing the amount of ornithine decarboxylase present are needed.

We have described the specific labelling of rat liver ornithine decarboxylase on incubation with radioactive DFMO (Pritchard *et al.*, 1981). DFMO is an enzyme-activated irreversible inhibitor of ornithine decarboxylase (Metcalf *et al.*, 1978; Bey, 1978). Binding of [^{14}C]DFMO to the enzyme should therefore provide a means of titrating the number of molecules of enzyme active in a tissue extract. In the present work this method has been used to determine this number in rodent tissues in a variety of physiological states. The results are in agreement with the hypothesis that changes in ornithine decarboxylase activity are brought about by changes in the number of active enzyme molecules.

Experimental

Materials

L-[1-^{14}C]Ornithine (57 Ci/mol) was purchased from NEN, Boston, MA, U.S.A. DL-[5-^{14}C]DFMO (60 Ci/mol) was obtained from Amersham–Searle, Arlington Heights, IL, U.S.A. Unlabelled DFMO was generously given by Merrell Dow, Cincinatti, OH, U.S.A. All other biochemical reagents were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Animals

Male Balb/c mice (about 20 g body wt.) were purchased from Jackson Laboratories, Bar Harbor, ME, U.S.A. Male Sprague–Dawley rats weighing 150–250 g and male Crl:CD-1 mice weighing about 30 g were purchased from Charles River Breeding Laboratories, Wilmington, MA, U.S.A. Animals were allowed standard laboratory chow and water *ad libitum* and maintained on a constant 12 h-light/12 h-dark controlled lighting schedule. Mice were treated with androgens by subcutaneous injection of a solution of testosterone propionate (4 mg/ml) at a dose of 100 mg/kg 3 days before death. Rats were treated with thioacetamide (150 mg/kg) by intraperitoneal injection of a solution of 75 mg/ml in 0.9% NaCl. Treatment with carbon tetrachloride was by intraperitoneal injection at a dose of 1.5 ml/kg. Cycloheximide was given at a dose of 1 mg/kg by intraperitoneal injection of a solution of 0.5 mg/ml in 0.9% NaCl. Control animals received the vehicle alone. Two-thirds partial hepatectomy was performed under light diethyl ether anaesthesia as previously described (Pösö & Pegg, 1982).

Preparation of tissue extracts

Animals were killed by cervical dislocation and tissues were homogenized in 2–3 vol. of buffer A (25 mM-Tris/HCl, pH 7.5, 2.5 mM-dithiothreitol, 0.1 mM-EDTA) at 0–4°C. After centrifugation at 100000 *g* for 45 min, the supernatant was taken. For mouse extracts, the supernatant was dialysed overnight against 200 vol. of buffer A and used directly. Rat liver extracts were fractionated by addition of $(NH_4)_2SO_4$. Proteins precipitated between 30 and 50% saturation with $(NH_4)_2SO_4$ were collected by centrifugation at 12000 *g* for 15 min, dissolved in one-quarter the original volume of buffer A and dialysed overnight against 50 vol. of buffer A. Approx. 90% of the original activity from the 100000 *g* supernatant could be recovered in this extract.

Measurement of ornithine decarboxylase activity

This was done by measuring the release of $^{14}CO_2$ from L-[1-^{14}C]ornithine as previously described (Pritchard *et al.*, 1981). The assay medium contained 0.4 mM-L-[1-^{14}C]ornithine (5 Ci/mol), 40 μM-pyridoxal phosphate, 2.5 mM-dithiothreitol and 25 mM-Tris/HCl, pH 7.5. A unit of enzyme activity released

1 nmol of CO_2 in a 30 min incubation at 37°C. Protein was determined by the method of Bradford (1976), with bovine serum albumin as standard. All assays were carried out under conditions in which activity was proportional to the amount of protein added and the time of incubation.

Binding of DFMO

Standard conditions for the binding of [5-^{14}C]-DFMO to protein consisted of incubation in buffer A containing 5 μM-[5-^{14}C]DFMO and 40 μM-pyridoxal phosphate. For liver extracts approx. 20 mg of protein was used in a total volume of 0.9 ml, and for mouse kidney extracts 3 mg of protein was used in a volume of 0.48 mol. After incubation at 37°C for 60 min, the protein was precipitated by the addition of 2 ml of 1 M-$HClO_4$ and centrifugation at 12000 *g* for 5 min. The pellet was washed with 2 × 2 ml of 1 M-$HClO_4$, once with 2 ml of ethanol/chloroform/ether (2:1:1, by vol.) and finally with ether. Each wash was followed by collection of the precipitate by centrifugation at 12000 *g* for 10 min. The protein pellet was dried in air for 2 h, dissolved in 1 ml of 0.1 M-NaOH at 100°C, cooled, mixed with 10 ml of ACS II scintillation fluid (Amersham–Searle) and counted for radioactivity in a liquid-scintillation spectrometer at an efficiency of 55% for the rat extracts and 67% for the mouse extracts (which contained less protein). Non-specific binding was determined with control incubations, which contained 30 mM-L-ornithine in addition to the normal compounds. This prevents the binding of DFMO to ornithine decarboxylase, and the amount of radioactivity present in such control tubes, which represents non-specific binding and the residual [^{14}C]-DFMO not removed by the washing procedure, was subtracted from that found when ornithine was absent to give the specific binding to ornithine decarboxylase. In some experiments the time of incubation and the concentration of DFMO was varied as indicated in the Figure legends.

Analysis by gel filtration and polyacrylamide-gel electrophoresis

Ornithine decarboxylase and [^{14}C]DFMO-labelled protein were chromatographed on a column (1.5 cm × 65 cm) of Ultrogel AcA 34 at a flow rate of 10 ml/h in buffer A. Fractions (1.5 ml) were collected and 0.5 ml portions were used for the determination of radioactivity after addition of 10 ml of ASC II scintillation fluid.

Polyacrylamide-gel electrophoresis under denaturing conditions was performed by mixing 50 μl portions of the labelled protein in buffer A with 150 μl of 2.5% (v/v) sodium dodecyl sulphate, 5% (v/v) 2-mercaptoethanol, 5 mM-dithiothreitol, 20% (v/v) glycerol and 0.001% Bromophenol Blue. The mixture was heated at 100°C for 2 min and the denatured protein was subjected to electrophoresis in 7.5%-acrylamide gels (Laemmli, 1970). The gels were calibrated by using marker proteins of known M_r (phosphorylase *a*, 94000; bovine serum albumin, 68000; ovalbumin, 43000; carbonic anhydrase, 30000). The gel was cut into 2 mm slices and the slices were placed into scintillation vials containing 1 ml of Protosol (NEN). Slices were allowed to dissolve overnight with mild agitation, and 10 ml of a toluene-based scintillation fluid (LSC-949, NEN) was added to each vial and the samples were counted for radioactivity.

Results

In order to establish standard conditions for the titration of rat tissue ornithine decarboxylase with [^{14}C]DFMO, the effect of concentration of the drug on enzyme activity and the incorporation of label into protein was tested in a standard assay involving 60 min exposure at 37°C in the assay buffer. Previous experiments had shown that this time is sufficient for complete inactivation and binding (Pritchard *et al.*, 1981). As shown in Fig. 1, there was an excellent agreement between the loss of enzyme activity and the incorporation of [^{14}C]DFMO into acid-insoluble material at all concentrations of

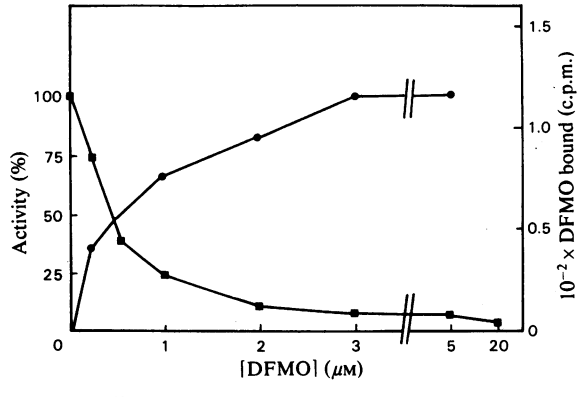

Fig. 1. *Effect of DFMO concentration on binding to rat ornithine decarboxylase and inactivation of the enzyme*

The enzyme used was obtained from rats treated with thioacetamide (150 mg/h, 24 h before death). The enzyme was incubated for 60 min at 37°C in the presence of the concentration of [5-^{14}C]DFMO shown, 25 mM-Tris/HCl, pH 7.5, 2.5 mM-dithiothreitol and 0.04 mM-pyridoxal phosphate. Samples were then used for the determination of ornithine decarboxylase activity (■) and DFMO bound to protein (●). The results shown are means of three determinations for each point. Activity is expressed as a percentage of that present in samples incubated without DFMO.

DFMO tested. A maximal binding and more than 90% loss of enzyme activity was achieved with DFMO concentrations of 3 μM or above. Therefore 5 μM was used in the experiments.

Rats were treated with a variety of agents known to induce hepatic ornithine decarboxylase activity. Liver extracts were prepared, and the ornithine decarboxylase activity was measured in one sample and the amount of [^{14}C]DFMO bound to protein under the standard assay conditions was determined in the other. As shown in Fig. 2, there was a constant relationship between the activity and the binding in all of these samples, which covered a 25-fold range of activities. It therefore appears that the enhanced ornithine decarboxylase activity brought about in the rat liver by carbon tetrachloride or thioacetamide or by partial hepatectomy is due to an increase in the amount of enzyme protein. The binding of DFMO to protein in the experiment of Fig. 2 occurred such that 26 fmol of drug was bound per unit of enzyme activity. The number of molecules of enzyme present per g of liver can therefore be calculated, assuming that only one molecule of drug is needed to inactivate one subunit of enzyme (Table 1). Even after stimulation, there is very little ornithine decarboxylase present in the liver, amounting to 0.02 pmol/mg of cytosolic protein. On the basis of a subunit M_r of 55000 for the liver enzyme (Pritchard *et al.*, 1981), there is only 1.1 ng of enzyme protein/mg of cytosolic protein (Table 1).

As shown in Fig. 3, the rapid disappearance of ornithine decarboxylase activity when protein synthesis was inhibited by cycloheximide is accompanied by loss of protein able to bind DFMO. Both activity and the drug-binding capacity were lost, with a half-life of about 15 min. Ornithine de-

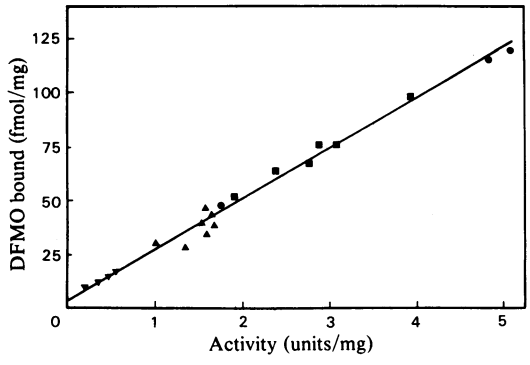

Fig. 2. *Correlation of rat liver ornithine decarboxylase activity and ability to bind DFMO*
Results are shown as means of three determinations from individual animals from rats treated with carbon tetrachloride (1.2 mg/kg 18 h before death, ■), thioacetamide (150 mg/kg 24 h before death, ●), or subjected to two-thirds partial hepatectomy (4 h before death, ▲) or given cycloheximide (1 mg/kg, 15 min before death and 4 h after partial hepatectomy, ▼).

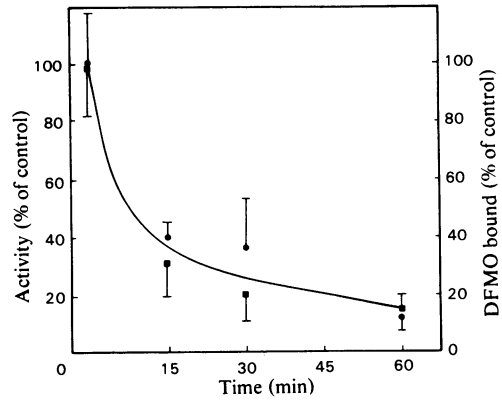

Fig. 3. *Effect of cycloheximide on ornithine decarboxylase activity and DFMO-binding capacity*
Rats were subjected to two-thirds partial hepatectomy and 4 h later were given 1 mg/kg doses of cycloheximide. They were killed at the time after giving cylcoheximide shown, and the enzyme activity (■) and DFMO-binding capacity (●) were determined. Results are shown as means ± S.D. for seven animals for each point.

Table 1. *Amounts of ornithine decarboxylase present in rat liver and mouse kidney*
Results are expressed as amounts per mg of protein in the ultracentrifuged supernatant fraction from the homogenates and per wet wt. of tissue.

	Ornithine decarboxylase activity		Ornithine decarboxylase protein	
Source	(units/mg of protein)	(units/g wet wt. of tissue)	(pmol/mg of protein)	(ng/mg of protein)
Mouse kidney (androgen-treated)	230	6440	3.2	171
Rat liver (24 h thioacetamide-treated)	0.8	57	0.02	1.1

Table 2. *Effect of 1,3-diaminopropane treatment on ornithine decarboxylase activity and binding of α-difluoromethylornithine to protein in rat liver extracts*

All rats were subjected to two-thirds partial hepatectomy and were treated with 1,3-diaminopropane immediately after operation or with saline and killed at the time later shown. Results are shown as means ± S.D. for five determinations.

Treatment	Time (h)	Ornithine decarboxylase activity (nmol/30min per mg)	DFMO binding (fmol/mg)
Control	4	1.45 ± 0.3	34.7 ± 6.7
1,3-Diaminopropane (1 mmol/kg)	4	0.26 ± 0.16	6.5 ± 3.0
1,3-Diaminopropane (1 mmol/kg)	6	1.45 ± 0.10	36.5 ± 3.5

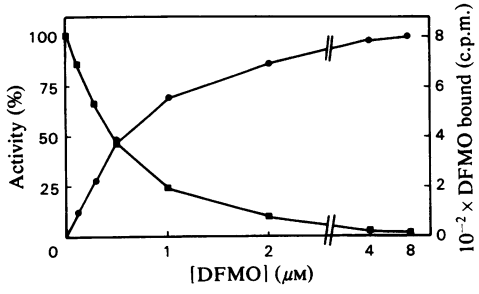

Fig. 4. *Effect of DFMO concentration on binding to mouse kidney ornithine decarboxylase and inactivation of the enzyme*

Enzyme isolated from kidneys of androgen-treated CD-1 strain mice was incubated for 60min as described for the rat liver extracts in Fig. 1. Results shown are means of three determinations for activity (■) and binding (●).

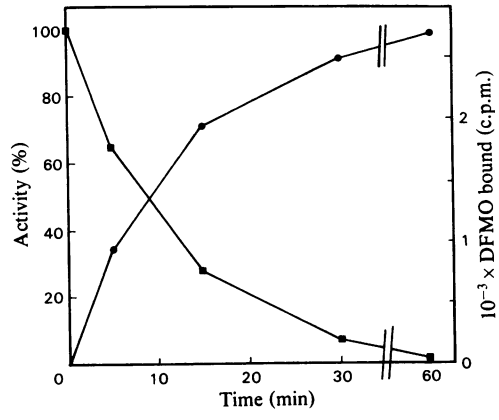

Fig. 5. *Time course of inactivation and binding of DFMO to mouse kidney ornithine decarboxylase*

The experiment was carried out as in Fig. 4 with 4.2 μM-[5-^{14}C]DFMO and with incubation for the time shown. ■, Activity; ●, binding.

carboxylase activity is also lost rapidly in mammalian tissues in response to the administration of a variety of α,ω-diamines, including 1,3-diaminopropane (Jänne *et al.*, 1978; Canellakis *et al.*, 1979). This decrease is transient (Pegg *et al.*, 1978). More than 80% of the ornithine decarboxylase activity in rat liver was lost within 4h after treatment with 1,3-diaminopropane, but by 6h full activity was restored (Table 2). The ability of liver extracts to incorporate [^{14}C]DFMO into protein was also lost in response to 1,3-diaminopropane and restored when the enzyme reappeared (Table 2).

The results summarized in Table 1 suggest that rat liver is not likely to be a good source of purified ornithine decarboxylase, since more than 1000 rats would be needed to obtain starting material for the purification of 1mg of enzyme, assuming a procedure giving 100% yield. Previous studies indicated that the mouse kidney had considerably higher enzyme activity, particularly after treatment with androgens (Pegg & McGill, 1979). As shown in Figs. 4 and 5, the ornithine decarboxylase present in mouse kidney extracts is also sensitive to inhibition by [^{14}C]DFMO, and there was again a good correlation between binding of the drug to protein and loss of enzyme activity. Fig. 4 shows that complete inactivation and maximal binding was achieved with concentrations of [^{14}C]DFMO of 4 μM. Fig. 5 shows a time-course of inactivation and binding with 4.2 μM-[^{14}C]DFMO. Inactivation was complete and binding was maximal at 60min. Therefore the standard conditions for titration of the rat liver enzyme are also appropriate for the mouse enzyme.

Fig. 6 shows ornithine decarboxylase and [^{14}C]-DFMO-binding activities of extracts prepared from two strains of mice, both control and androgen-treated. There was again an excellent correlation between the two parameters over a range of samples which varied in ornithine decarboxylase activities by 100-fold. Activity of ornithine decarboxylase and the amount of enzyme protein present were increased substantially by androgen treatment in both strains, to about the same extent. However, the basal value was lower in the Balb/c strain, which was therefore stimulated to a greater extent. The inactivation of 1 unit of mouse kidney ornithine decarboxylase

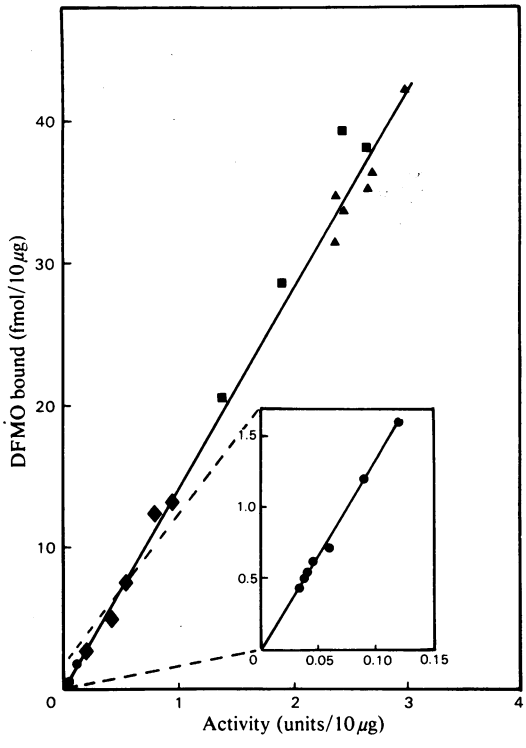

Fig. 6. *Correlation of mouse kidney ornithine decarboxylase activity and ability to bind DFMO*

Results shown are means of three determinations on individual animals, for control (♦) and androgen-treated CD-1 mice (■), and control (●) and androgen-treated Balb/c mice (▲). The inset expands results obtained with the lower activities.

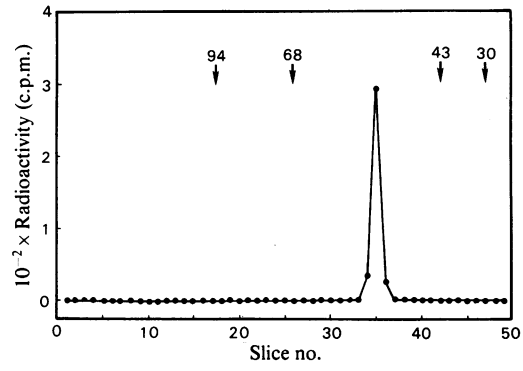

Fig. 7. *Polyacrylamide-gel electrophoresis of DFMO-labelled mouse ornithine decarboxylase*

The enzyme was inactivated with [^{14}C]DFMO and dialysed against 25mM-Tris/HCl (pH 7.5)/2.5mM-dithiothreitol/0.1mM-EDTA to remove unbound drug, and 50μl of the dialysed protein solution was subjected to polyacrylamide-gel electrophoresis under denaturing conditions as described in the Experimental section. The gel was sliced into 2mm slices and the radioactivity determined as described. The positions of marker proteins of known M_r ($\times 10^{-3}$) are indicated.

required 14fmol of drug. This value is approximately half of that needed to inactivate the rat liver enzyme, but, as shown in Figs. 7 and 8, the mouse enzyme is of a similar size, having a subunit M_r of about 55000 and an apparent M_r of the native enzyme of just over 100000. Fig. 8 also shows that the protein labelled by reaction with [^{14}C]DFMO is co-eluted with mouse ornithine decarboxylase when chromatographed on a gel-filtration column. These results therefore suggest that the mouse ornithine decarboxylase has a higher catalytic-centre activity than the rat liver enzyme. The results could also be explained if inhibition of the rat liver enzyme required more than one molecule of inhibitor to be bound for inactivation, but this is unlikely.

The amount of ornithine decarboxylase present in mouse kidney extracts was calculated from the [^{14}C]DFMO-binding data, and is shown in Table 1. Androgen-treated mouse kidneys contain over 100 times more ornithine decarboxylase than does

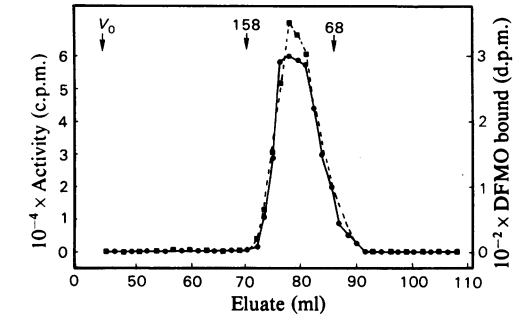

Fig. 8. *Gel-filtration chromatography of native mouse kidney ornithine decarboxylase and DFMO-labelled protein*

The enzyme was inactivated as in Fig. 7, and the final preparation, containing 5800d.p.m. bound to protein, was mixed with native enzyme equivalent to 500 units and the mixture chromatographed on a column (1.5cm × 65cm) of Ultrogel AcA 34 eluted at a flow rate of 10ml/h. Fractions (1.5ml) were collected and radioactivity (●) present or activity (■) was determined. Protein standards used in molecular-weight calibration were aldolase (158000), bovine serum albumin (68000) and cytochrome *c* (12500), which were eluted at 70, 86 and 116ml respectively. Blue Dextran was used in the determination of the void volume (V_0; 45ml).

thioacetamide-treated rat liver, and are therefore a better potential source for purification of the enzyme.

Discussion

The present results show that over a wide range of ornithine decarboxylase activities under various physiological stimuli there is an excellent correlation between the ability to bind radioactive DFMO and the enzymic activity. In fact, the ratio between these parameters was more constant in animals treated with the same agent than was the activity itself. Figs. 2 and 6 show that there were quite substantial variations in activity in animals receiving the same stimulus, but that those with the highest activity also had the highest capacity to bind DFMO. The results are consistent with the hypothesis that changes in activity are brought about by changes in the amount of enzyme protein.

The use of DFMO binding to quantify the amount of ornithine decarboxylase present in the tissues depends on the stability of the bond between the drug and the enzyme and the specificity of bond formation. When measured under the conditions described here, there was a negligible increase in binding when all of the ornithine decarboxylase activity was gone, and the correlation between binding and activities discussed above argues strongly in favour of this specificity. Also, as shown in Figs. 7 and 8 for the mouse enzyme, and previously for the rat enzyme (Pritchard *et al.*, 1981) only one peak of labelled material was observed when the DFMO-labelled material was separated by gel filtration or polyacrylamide-gel electrophoresis. The stability of the labelled protein is such that no change in labelling was seen on treatment of the protein with detergents, heating in 10% $HClO_4$ and prolonged dialysis. Furthermore, the extent of binding to protein in the present procedure involving acid precipitation and washing was the same as that obtained with milder and more rapid procedures to remove the unbound drug, such as gel filtration, $(NH_4)_2SO_4$ precipitation or dialysis (Pritchard *et al.*, 1981; Pegg *et al.*, 1981). There is therefore no good reason to suppose that some of the DFMO becomes dissociated from the enzyme after inactivation, leaving the enzyme inactivated but unlabelled. The results shown in Table 1 for the amounts of ornithine decarboxylase present in liver and kidney may be slightly overestimated, since it is assumed that the enzyme binds one molecule of drug for each subunit when inactivated, whereas it could bind only one molecule per dimer, but these are likely to be close to the correct values. The predicted specific activity of homogeneous rat liver ornithine decarboxylase from these results would be 25 μmol of CO_2/min per mg, which is higher than values in the literature (Obenrader & Prouty, 1977; Pegg & McGill, 1979; Kitani & Fujisawa, 1981), but agrees with the report by Kitani & Fujisawa (1981) that enzyme having a specific activity of 1 μmol/min per mg was not homogeneous. More recently, Kameji *et al.* (1981) have purified rat liver ornithine decarboxylase to approximately the predicted specific activity. The predicted specific activity of the mouse enzyme is 50 μmol of CO_2/min per mg, which is also more than that so far obtained for the enzyme from mouse tissues and cells (Weiss *et al.*, 1981; Persson, 1981), but we have subsequently purified the enzyme from mouse kidney to this value (Seely *et al.*, 1982).

It was suggested that calf liver is a suitable material for the purification of eukaryotic ornithine decarboxylase (Haddox & Russell, 1981). However, perhaps owing to the problems of optimizing the dose of thioacetamide used to induce the enzyme in the calf, the starting specific activity was only about one-quarter of that obtained in the induced rat liver. Moreover, it appears improbable that the final preparation, which had a specific activity of only 0.29 μmol/min per mg (Haddox & Russell, 1981), was homogeneous unless there are very large differences between the bovine and rodent enzymes. In any case, the androgen-induced mouse kidney contains 600 times more enzyme activity than bovine liver, which more than compensates for the large size, which appears to be the sole merit of the latter source.

The binding of DFMO to ornithine decarboxylase provides a useful method for assessing the purity of the enzyme and should prove valuable in confirming reports of post-translational modifications to ornithine decarboxylase, which may have been performed with preparations that were not homogeneous (Mitchell, 1981; Russell, 1981; Atmar & Kuehn, 1981). However, the titration with DFMO may not be suitable for investigating the inhibition of ornithine decarboxylase activity by the protein inhibitor termed 'antizyme' (Heller *et al.*, 1976; Canellakis *et al.*, 1979; Heller & Canellakis, 1981). This inhibitor acts in a non-competitive fashion (Heller *et al.*, 1976), and it might be expected that the binding of DFMO would proceed even in the presence of the inhibitor, albeit at a slower rate. However, as shown in Table 1, the loss of enzyme activity in response to 1,3-diaminopropane, which is a known inducer of antizyme at the dose used (Kallio *et al.*, 1979), was accompanied by a parallel loss of DFMO-binding activity. This may indicate that the enzyme–antizyme complex is not available to bind DFMO, or that the enzyme is further degraded after complexing with antizyme. More recently, Mitchell *et al.* (1981) have also noted that a form of ornithine decarboxylase not active under physiological conditions in *Physarum* is also not able to bind DFMO. Therefore titration with DFMO provides only an

estimate of the number of molecules of ornithine decarboxylase that are active.

The binding of DFMO to ornithine decarboxylase provides a valuable new tool with which to study the amount of enzyme and its purification. This tool would have even greater value if highly specific and high-affinity antibodies to the protein were used to complement these studies, since a radioimmunoassay for the protein could then be set up. The availability of homogeneous preparations of ornithine decarboxylase isolated from mouse kidney (Seely *et al.*, 1982) should help in the production of such antibodies. The labelling with DFMO can also be used for autoradiographic localization of the enzyme (Pegg *et al.*, 1982). The specific activity of the DFMO used in the present experiments is not sufficiently high for quantification or localization of the enzyme in unstimulated tissues, but preparation of suitable material labelled with ^3H should present no difficulty.

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References

Atmar, V. J. & Kuehn, G. D. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5518–5522

Bachrach, U. (1980) in *Polyamines in Biomedical Research* (Gaugas, J. M., ed.), pp. 81–108, John Wiley, New York

Bey, P. (1978) in *Enzyme-Activated Irreversible Inhibitors* (Seiler, N., Jung, M. J. & Koch-Weser, J., eds.), pp. 27–41, Elsevier/North-Holland, Amsterdam

Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254

Canellakis, E. S., Heller, J. S., Kyriakidis, D. A. & Chen, K. Y. (1978) in *Advances in Polyamine Research* (Campbell, R. A., Morris, D. R., Bartos, D., Daves, G. D. & Bartos, F., eds.), vol. 1, pp. 17–30, Raven Press, New York

Canellakis, E. S., Viceps-Madore, D., Kyriakidis, D. A. & Heller, J. S. (1979) *Curr. Top. Cell. Regul.* **15**, 155–202

Canellakis, Z. N. & Theoharides, T. C. (1976) *J. Biol. Chem.* **251**, 4436–4441

Haddox, M. & Russell, D. H. (1981) *Biochemistry* **20**, 6721–6729

Heller, J. S. & Canellakis, E. S. (1981) *J. Cell. Physiol.* **107**, 209–217

Heller, J. S., Fong, W. F. & Canellakis, E. S. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1858–1862

Hölttä, E. (1975) *Biochim. Biophys. Acta* **399**, 420–427

Jänne, J., Pösö, H. & Raina, A. (1978) *Biochim. Biophys. Acta* **473**, 241–293

Kallio, A., Löfman, M., Pösö, H. & Jänne, J. (1977) *FEBS Lett.* **79**, 195–199

Kallio, A., Löfman, M., Pösö, H. & Jänne, J. (1979) *Biochem. J.* **177**, 63–69

Kameji, T., Murakami, Y., Fujita, K., Noguchi, T. & Hayashi, S. (1981) *Med. Biol.* **59**, 296–300

Kitani, T. & Fujisawa, H. (1981) *Eur. J. Biochem.* **119**, 177–181

Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685

Metcalf, B. W., Bey, P., Danzin, C., Jung, M. J., Casara, P. & Vevert, J. P. (1978) *J. Am. Chem. Soc.* **100**, 2551–2553

Mitchell, J. L. A. (1981) in *Advances in Polyamine Research* (Calderera, C. M., Zappia, V. & Bachrach, U., eds.), vol. 3, pp. 15–26, Raven Press, New York

Mitchell, J. L. A., Yingling, R. A. & Mitchell, G. K. (1981) *FEBS Lett.* **131**, 305–309

Morris, D. R. & Fillingame, R. H. (1974) *Annu. Rev. Biochem.* **43**, 303–325

Obenrader, M. F. & Prouty, W. F. (1977) *J. Biol. Chem.* **252**, 2866–2872

Pegg, A. E. & McGill, S. (1979) *Biochim. Biophys. Acta* **568**, 416–427

Pegg, A. E. & Williams-Ashman, H. G. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R. & Marton, L. J., eds.), pp. 3–42, Marcel Dekker, New York

Pegg, A. E., Conover, C. & Wrona, A. (1978) *Biochem. J.* **170**, 651–660

Pegg, A. E., Matsui, I., Seely, J. E., Pritchard, M. L. & Pösö, H. (1981) *Med. Biol.* **59**, 327–334

Pegg, A. E., Seely, J. & Zagon, I. (1982) *Science* in the press

Persson, L. (1981) *Acta Chem. Scand. Ser. B* **35**, 451–459

Pösö, H. & Pegg, A. E. (1982) *Biochim. Biophys. Acta* **696**, 179–186

Pritchard, M. L., Seely, J. E., Pösö, H., Jefferson, L. S. & Pegg, A. E. (1981) *Biochem. Biophys. Res. Commun.* **100**, 1597–1603

Russell, D. H. (1980) *Pharmacology* **20**, 117–129

Russell, D. H. (1981) *Biochem. Biophys. Res. Commun.* **99**, 1167–1172

Russell, D. H. & Durie, B. G. M. (1978) *Prog. Cancer Res. Ther.* **8**, 1–178

Seely, J. E., Pösö, H. & Pegg, A. E. (1982) *Biochemistry* in the press

Tabor, C. W. & Tabor, H. (1976) *Annu. Rev. Biochem.* **45**, 285–306

Theoharides, T. C. & Canellakis, Z. N. (1976) *J. Biol. Chem.* **251**, 1781–1784

Weiss, J. M., Lembach, K. J. & Boucek, R. J. (1981) *Biochem. J.* **194**, 229–239