Mechanism of Stimulation of Ornithine Decarboxylase Activity in Transformed Mouse Fibroblasts†

Bradley G. Erwin, James E. Seely,‡ and Anthony E. Pegg*

ABSTRACT: Stimulation of confluent serum-starved SV-3T3 cells by serum produces a transient enhancement of ornithine decarboxylase activity which fluctuates at least 20-fold over a 12-h period. The mechanism by which this fluctuation is produced was investigated. Two techniques for assaying the amount of enzyme protein in these cells were utilized: radioimmunoassay and titration with α-(difluoromethyl)[5-^{3}H]ornithine. The radioimmunoassay was carried out by using a specific antiserum prepared in rabbits against homogeneous mouse kidney ornithine decarboxylase and by using α-(difluoromethyl)[5-^{3}H]ornithine-labeled kidney ornithine decarboxylase as the tracer ligand. An exact correlation between the amount of enzyme protein and the amount of enzyme activity was seen during the rise and fall of ornithine decarboxylase activity after serum stimulation. Similarly, the amount of protein which was labeled covalently by reaction with α-(difluoromethyl)[5-^{3}H]ornithine (a specific enzyme-activated irreversible inhibitor) correlated with the enzyme activity. Investigation of the protein labeled in this way by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate indicated that no change in the size of the protein which had a molecular weight of 53 000 occurred during this time period. These results indicate the alteration in ornithine decarboxylase activity can be accounted for entirely by changes in the amount of enzyme protein rather than by posttranslational modifications, activators, or inhibitors.

L-Ornithine decarboxylase, the first enzyme in the polyamine biosynthetic pathway, shows remarkable fluctuations in activity in response to many different stimuli. Increases in the activity of this enzyme amounting to 10–100-fold have been observed within a few hours of stimulation with hormones, toxins, other growth-promoting factors, and many drugs (Morris & Fillingame, 1974; Jänne et al., 1978; McCann, 1980; Russell, 1980). Such increases are also transient, and an equally rapid fall in enzyme activity occurs when the peak is passed. The underlying mechanism for these changes is not well understood. L-Ornithine decarboxylase has a very rapid rate of turnover in mammalian cells (Russell, 1980; Pegg & Williams-Ashman, 1981; Seely et al., 1982a), and, therefore, alterations in the activity could be mediated by changes in the absolute amount of enzyme protein (Berlin & Schimke, 1965). However, the unusual extent and rapidity of these changes in activity have led to suggestions that the enzyme may also be regulated by posttranslational modifications, or by interaction with stimulatory or inhibitory macromolecules. A wide variety of such candidates have been postulated and supported by suggestive, but not fully conclusive, experimental evidence. These include phosphorylation (Atmar & Kuehn, 1981), transglutamination (Russell, 1981), formation of less active aggregates in response to the absence of sulfhydryl reducing agents (Jänne & Williams-Ashman, 1971), changes in the binding of the pyridoxal phosphate cofactor (Clark & Fuller, 1976; Mitchell, 1981; Mitchell & Mitchell, 1982; Mitchell et al., 1982), and complexation with an inhibitory protein termed antizyme (Canellakis et al., 1979, 1981) and with an activating protein (Canellakis et al., 1981; Fujita et al., 1982).

The major reason that this situation is still unclear is the small amount of ornithine decarboxylase present in mammalian cells. Even in the androgen-induced mouse kidney, this enzyme amounts to only 0.01% of the soluble protein, and this tissue is easily the best source of the enzyme, containing 100–200 times greater levels than those present in most other cells even after maximal stimulation (Seely et al., 1982b,c). In unstimulated cells, ornithine decarboxylase is frequently less than 0.00001% of the cellular protein [equivalent to less than 10 000 molecules per cell (Pritchard et al., 1981; Pegg et al., 1981, 1982a)]. Therefore, it is not a simple procedure to isolate ornithine decarboxylase from stimulated and unstimulated cells and compare the enzyme by standard biochemical techniques. In the present work, we have studied the changes in ornithine decarboxylase in SV-3T3 cells after stimulation of quiescent, serum-starved cells with fresh serum. Such stimulation induces a rapid rise and subsequent fall in the enzyme activity in these and other cells (Lembach, 1974; Hogan et al., 1974; Clark, 1974; Clark & Duffy, 1976; McCann, 1980; Bethell & Pegg, 1979). The absolute amount of enzyme protein present in these cells was studied by two techniques: titration with the enzyme-activated irreversible inhibitor α-(difluoromethyl)ornithine (Pritchard et al., 1981; Seely et al., 1982c) and radioimmunoassay. These methods provide independent assay procedures for determination of the amount of enzyme protein with sufficient sensitivity to quantitate the amount present in cell cultures. The results indicate that the fluctuation in ornithine decarboxylase activity seen in response to serum is accounted for solely by changes in the amount of enzyme protein.

Materials and Methods

Materials. L-[1-^{14}C]Ornithine (57 Ci/mol) and α-DL-(difluoromethyl)[5-^{3}H]ornithine (17.2 or 22.5 Ci/mol) were purchased from New England Nuclear (Boston, MA). Unlabeled α-(difluoromethyl)ornithine was a generous gift from Merrell-Dow (Cincinnati, OH). Protein A bacterial adsorbent was purchased from Miles-Yeda (Rehovot, Israel). Tissue culture dishes were from Falcon Plastics (Cockeysville, MD); sera and medium were from Flow Laboratories (McLean, VA). Liquid scintillation cocktail (ACS-II) was purchased from Amersham/Searle (Arlington Heights, IL). All other biochemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

† From the Department of Physiology and Specialized Cancer Research Center, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033. *Received February 28, 1983.* This investigation was supported by Grants CA-18138 and 1 P30 CA-18450 from the National Institutes of Health.

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Cell Culture and Preparation of Cell Extracts. Stock and experimental cultures of SV-40 virus transformed mouse embryo fibroblasts were grown in Dulbecco's modified Eagle's medium supplemented with 3% horse serum, 2% fetal bovine serum, 36 μM $NaHCO_3$, penicillin (9 units/mL), streptomycin (9 μg/mL), and 2 μM *n*-butyl *p*-hydroxybenzoate. All cultures were grown in a humidified atmosphere of 10% CO_2 at 37 °C. Stock and experimental cultures were seeded at 4×10^5 cells per 10-cm diameter tissue culture dish. Stock cultures were subcultured by trypsinization with a 0.25% (w/v) solution and replated in fresh growth medium every 3 days. Experimental cultures were allowed to proliferate for 3 days after which the medium was removed and replaced with growth medium devoid of serum. Twenty-four hours later, ornithine decarboxylase activity was stimulated by adjusting the medium to contain 10% fetal bovine serum. This juncture was designated zero time, and cells were extracted for enzyme at the times indicated. The stimulatory medium was removed, and the cell surface was washed twice with 5 mL of phosphate-buffered saline. The washes and all subsequent extraction procedures were conducted at 4 °C. The cells were lysed in 1 mL of a hypotonic harvest buffer consisting of 25 mM tris(hydroxymethyl)aminomethane (Tris) (pH 7.5), 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 2.5 mM dithiothreitol. The resulting extract was snap-frozen in liquid nitrogen. This was followed by centrifugation at 17000*g* for 30 min. The supernatant was removed and used as extract for the assays of enzyme activity and protein. Protein was determined by the method of Bradford (1976) with bovine serum albumin as standard.

Assay of Ornithine Decarboxylase Activity. Activity was measured by following the production of $^{14}CO_2$ as previously described (Pritchard et al., 1981). The assays contained 0.4 mM L-[1-^{14}C]ornithine (2.5 Ci/mol), 50 mM Tris-HCl, pH 7.5, 40 μM pyridoxal phosphate, 0.1 mM EDTA, and 2.5 mM dithiothreitol in a total volume of 0.25 mL. One unit of enzyme activity was defined as that producing 1 nmol of CO_2 in 30 min at 37 °C.

Titration with α-(Difluoromethyl)[5-^{3}H]ornithine. The extracts were incubated with 0.35 μM α-(difluoromethyl)[5-^{3}H]ornithine (8 μCi/mL with the drug at a specific activity of 22.5 Ci/mmol and 6 μCi/mL with 17.2 Ci/mmol specific activity), 40 μM pyridoxal phosphate, 100 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, and 2.5 mM dithiothreitol for 5 h at 37 °C or for the time indicated. (Aliquots were used for the determination of the remaining enzyme activity where shown.) The reaction was terminated by the addition of 10 volumes of 1 M perchloric acid containing 0.1% (w/v) α-(difluoromethyl)ornithine. After 1 h at 4 °C, 3 mg of carrier protein (bovine serum albumin) was added, and the samples were sonicated by using a Heat Systems Ultra Sonics (Plainview, NY) water bath sonicator. The pellet was collected by centrifugation at 35000*g* for 15 min and washed 3 times by resuspending in 2 mL of 1 M perchloric acid and sonicating and centrifuging as described above. After three washes, no radioactivity was present in the supernatant, and the pellet was dissolved in 1 mL of 0.1 M NaOH, mixed with 10 mL of liquid scintillation cocktail, and counted.

Extraction, Titration, and Purification of Mouse Kidney Ornithine Decarboxylase. Mouse kidney extracts containing ornithine decarboxylase were prepared as described by Seely et al. (1982a). Binding of α-(difluoromethyl)[5-^{3}H]ornithine to the crude (Table I) or purified enzyme was carried out as previously described (Seely et al., 1982c). The enzyme was purified to homogeneity (Seely et al., 1982b), and antibodies were raised in rabbits (Seely & Pegg, 1983).

Table I: Incorporation of Radioactivity into Protein and Loss of Ornithine Decarboxylase Activity when Mouse Kidney Extracts Were Incubated with α-(Difluoromethyl)[5-^{3}H]ornithine[a]

amount and source of extract from mouse kidney	time (min)	activity of ornithine decarboxylase (units)	radioactivity incorporated into protein (dpm)	
			total binding	nonspecific binding
0.1 mg, androgen stimulated	0	27		
0.1 mg, androgen stimulated	20	7.6	21 857	1 037
0.1 mg, androgen stimulated	60	1.1	29 950	1 083
0.1 mg, androgen stimulated	120	0.3	32 862	1 329
2 mg, female	0	1		
2 mg, female	60	<0.05	19 179	17 300

[a] Mouse kidney extracts were incubated for up to 2 h with 2.5 μM α-(difluoromethyl)[5-^{3}H]ornithine in a total volume of 0.3 mL. The total binding of the drug to protein and the nonspecific binding (which also includes any free drug not removed by the washing procedure) were determined as described by Seely et al. (1982c).

Radioimmunoassay of Ornithine Decarboxylase. This was carried out essentially as described by Seely & Pegg (1983). The standards or samples to be assayed (containing at least 3 units of ornithine decarboxylase activity) were incubated with 0.05 mL of a 1:200 dilution of antiserum in 100 mM Tris-HCl, pH 7.5, 0.5 mM dithiothreitol, 0.5 mM EDTA, and 0.02% Brij 35 in a total volume of not more than 1.75 mL for 30 min at room temperature. Then 0.05 mL of a solution in the same buffer of α-(difluoromethyl)[5-^{3}H]ornithine-labeled ornithine decarboxylase was added and the incubation continued for an additional 30 min. Bacterial protein A adsorbent (25 μL of a 10% suspension) was added, and after the solution was shaken for 90 min, the samples were centrifuged at 15000 rpm in an Eppendorf microfuge for 1 min. The pellet was washed twice by resuspension in 0.75 mL of 150 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, and 0.02% Brij 35 and centrifuged. The washed pellet was resuspended in 0.2 mL of distilled water, and the radioactivity present was determined after addition of 10 mL of ACS-II scintillation cocktail. The amount of antiserum used was such that approximately 50% of the added tracer ligand was precipitated in the absence of any unlabeled ornithine decarboxylase. The results of the standard curve using mouse kidney ornithine decarboxylase were plotted as the logit of the percentage of the binding found compared to that when no standard was added against the log of the ornithine decarboxylase protein present (Hunter, 1978). The amount of protein in the extracts from the SV-3T3 cells was then read off from the standard curve. A standard curve was carried out at the same time as all experimental measurements, and the volume was the same as for the assays carried out at that time.

Polyacrylamide Gel Electrophoresis of Labeled Ornithine Decarboxylase. After incorporation of (difluoromethyl)[5-^{3}H]ornithine for 5 h as described above, the samples were dialyzed overnight against 100 volumes of 50 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 1 mM dithiothreitol, 0.02% Brij 35, and 0.05% unlabeled α-(difluoromethyl)ornithine. The labeled enzyme was then precipitated by adding 0.5 μL (0.05 mL of a 1:100 dilution) of antiserum to 1.5 mL of the dialyzed extract. After 90 min at room temperature, 25 μL of protein A bacterial adsorbent (10% suspension) was added, and the

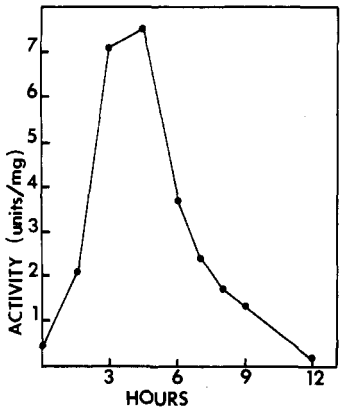

FIGURE 1: Stimulation of ornithine decarboxylase activity by serum. Confluent (3-day) cultures of SV-3T3 cells were shifted to a medium lacking serum for 24 h. Fresh fetal bovine serum was then added to a final concentration of 10% and ornithine decarboxylase activity assayed at various times thereafter as shown.

extracts were incubated for a further 90 min. The pellet was collected by centrifugation at 15 000 rpm for 1 min, washed 4 times in 50 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 1 mM dithiothreitol, 0.02% Brij 35, and 0.05% (w/v) α-(difluoromethyl)ornithine, and heated at 100 °C for 5 min in 0.1–0.2 mL of a solubilizing solution. This solution contained 2.5% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 5 mM dithiothreitol, 20% glycerol, and 0.001% bromophenol blue. The solubilized pellet was centrifuged as above to remove the bacterial membranes and the supernatant subjected to electrophoresis on 10% polyacrylamide gels as previously described (Seely et al., 1982b). In some experiments, the labeled protein was precipitated with 1 M perchloric acid as described above instead of with the antibody. These precipitates were solubilized in the same solution and gave the same band on electrophoresis on polyacrylamide gels, but this method could only be used for the samples containing larger amounts of ornithine decarboxylase because all of the protein in the extract had to be loaded onto the gels. These were, therefore, overloaded with protein except where ornithine decarboxylase was near to peak levels.

Results

Addition of serum to confluent serum-starved cultures of SV-3T3 resulted in a transient 20-fold rise in the activity of ornithine decarboxylase as previously reported (Lembach, 1974; Bethell & Pegg, 1979). A typical experiment is shown in Figure 1; the activity peaked at 3–4.5 h after serum stimulation and declined rapidly over the next 6 h, returning to control values by 12 h. Two techniques for determination of the amount of ornithine decarboxylase protein were used to investigate whether these fluctuations in activity were due to alterations in the amount of protein or to posttranslational changes resulting in its activation/deactivation.

The first technique used was to attempt to titrate the number of ornithine decarboxylase molecules by using α-(difluoromethyl)ornithine. This drug is an enzyme-activated, irreversible inhibitor which forms a covalent bond to the enzyme. It has been shown by using the purified enzyme from rat liver (Kameji & Hayashi, 1982) or mouse kidney (Seely et al., 1982b,c) that inactivation is accompanied by the incorporation of stoichiometric amounts of the drug per active subunit of the enzyme. Measurment of the conversion of α-(difluoromethyl)[5-^{14}C]ornithine (60 Ci/mol) into a protein-bound form has, therefore, been used to determine the number of molecules of the enzyme present in extracts of androgen-stimulated mouse kidney and thioacetamide-stimulated rat liver (Pritchard et al., 1981; Seely et al., 1982a,c). However, this technique has a limited sensitivity since only 2–4 dpm are incorporated per unit of enzyme activity. We, therefore, attempted to increase the sensitivity of this titration by using α-(difluoromethyl)[5-^{3}H]ornithine of high specific activity for the reaction. As shown in Table I, when crude mouse kidney extracts (from androgen-treated male mice) were incubated in the presence of 2.5 μM α-(difluoromethyl)[5-^{3}H]ornithine, there was a complete loss of activity and a substantial incorporation of radioactivity into protein. Nonspecific binding of the labeled drug to protein in this system (which was measured by addition of 30 mM L-ornithine to prevent inactivation of ornithine decarboxylase) was negligible, amounting to only 1300 dpm in 2-h incubation, whereas the total incorporation was 33 000 dpm in this time. However, these extracts contained 27 units of ornithine decarboxylase in about 100 μg of protein. When extracts from female mouse kidneys which contained only about 1 unit of activity in 2 mg of protein were incubated in a similar system for 60 min, the nonspecific incorporation was increased to 17 000 dpm, and the total was only about 19 000 dpm. Therefore, it appears that the nonspecific incorporation was proportional to the protein concentration and to the time of incubation and that this places limitations on the sensitivity of the method which cannot be overcome by using an inhibitor of high specific activity. In order to test whether this system was suitable for titration of the enzyme in extracts from SV-3T3 cells, we used a lower concentration of radioactive α-(difluoromethyl)[5-^{3}H]ornithine for economic reasons and to attempt to reduce the nonspecific binding. As shown in Figure 2, incubation of extracts containing 6 units of enzyme in about 1.7 mg of total protein with 0.35 μM α-(difluoromethyl)[5-^{3}H]ornithine in a total volume of 0.8 mL gave satisfactory results. Approximately 87% of the enzyme activity was lost in 5 h under these conditions (and less than 10% was lost when no inhibitor was included). The nonspecific binding was assayed by using either ovalbumin as the protein or an extract from SV-3T3 cells in which all ornithine decarboxylase activity had been removed by reaction with unlabeled α-(difluoromethyl)ornithine. There was no significant difference between the results for nonspecific binding obtained with these materials. The total binding to the sample containing ornithine decarboxylase activity was at all times more than 7-fold greater than the nonspecific binding. At each time point, there was an excellent correlation between

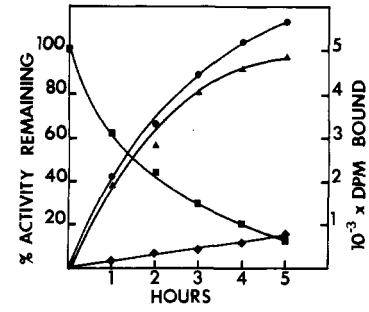

FIGURE 2: Titration of ornithine decarboxylase activity in extracts of SV-3T3 cells with α-(difluoromethyl)[5-^{3}H]ornithine. The extract containing 6 units of activity (1.7 mg of protein) was incubated with 0.35 μM α-(difluoromethyl)[5-^{3}H]ornithine in a total volume of 0.8 mL for the time shown. The activity remaining (■) and the radioactivity incorporated into protein (●) were then determined. The nonspecific incorporation of radioactivity into protein (♦) was measured by using the same amount of protein but replacing the extract with either ovalbumin or an SV-3T3 cell protein extract in which all ornithine decarboxylase had been inactivated. The specific binding to ornithine decarboxylase (▲) was calculated by subtracting the nonspecific from the total incorporation.

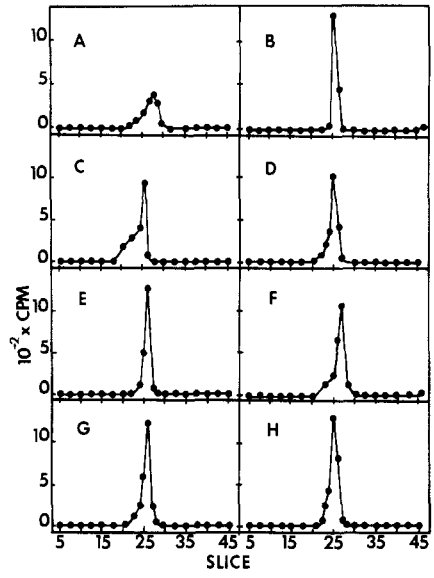

FIGURE 3: Polyacrylamide gel electrophoresis of protein labeled with α-(difluoromethyl)[5-^{3}H]ornithine. Electrophoresis was carried out as described under Materials and Methods with extracts isolated from cells stimulated 1.5 (A), 3 (B), 4.5 (C), 6 (D), 7 (E), 8 (F), and 9 h (G) prior to harvest. The amount of total protein loaded was varied to ensure at least 500 cpm in the ornithine decarboxylase. Panel H shows a result with standard mouse kidney enzyme.

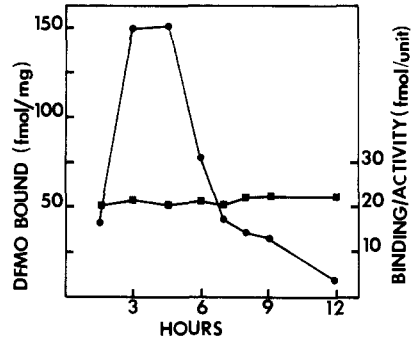

FIGURE 4: Effect of serum stimulation on specific binding of α-(difluoromethyl)[5-^{3}H]ornithine by extracts of SV-3T3 cells. Extracts were prepared as in Figure 1, and the specific binding of the drug was measured as in Figure 2 and described under Materials and Methods. Results are plotted as the amount of α-(difluoromethyl)[5-^{3}H]ornithine bound per milligram of protein at the times shown (●) and as the ratio (■) of this value to the activity of ornithine decarboxylase initially present in the extracts.

the amount of activity lost and the radioactivity incorporated into ornithine decarboxylase as determined by subtracting the nonspecific binding from the total. Even with extracts containing lower amounts of ornithine decarboxylase, a substantial increment over the nonspecific binding was obtained until the activity was less than 0.5 unit/mg. Therefore, these conditions were used to determine the amount of ornithine decarboxylase titratable with α-(difluoromethyl)[5-^{3}H]ornithine.

The results obtained with such titrations are shown in Figures 3 and 4. As shown in Figure 3, a single band was labeled with this drug when the protein was analyzed on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. This band had the same mobility, which corresponded to a molecular weight of 53 000, irrespective of the time after stimulation with serum. Therefore, the alterations in ornithine decarboxylase activity are not accompanied by any substantial change in the size of the protein. Figure 4 shows the effect of serum stimulation on the amount of protein reacting with α-(difluoromethyl)[5-^{3}H]ornithine in SV-3T3 cells. It can be seen that this varied exactly in parallel

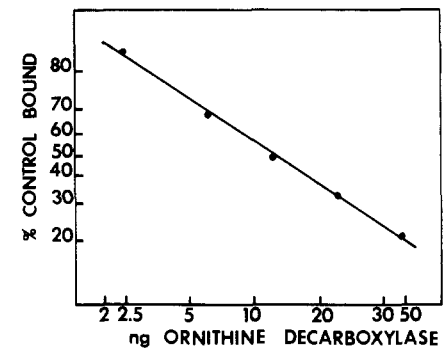

FIGURE 5: Radioimmunoassay standard curve for ornithine decarboxylase. The assay was carried out as described under Materials and Methods and a standard curve set up by using the amounts of mouse kidney ornithine decarboxylase shown. The results are expressed as the percentage of the tracer ligand bound in the absence of any ornithine decarboxylase, and the logit of this value is plotted against the log of the ornithine decarboxylase protein concentration added.

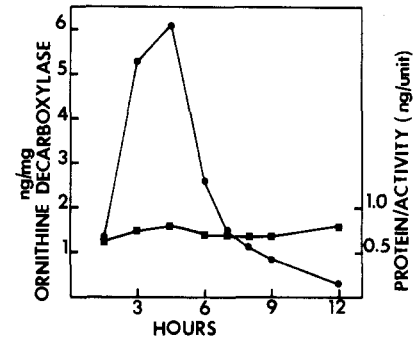

FIGURE 6: Effect of serum stimulation on amount of ornithine decarboxylase protein in SV-3T3 cells. The extracts were prepared as in Figure 1, and the amounts of ornithine decarboxylase protein present (●) were determined by radioimmunoassay as in Figure 5. The ratio (■) of this value to the activity of ornithine decarboxylase present in the extracts is also shown.

with the change in enzyme activity seen in Figure 1. Therefore, the alterations in activity appear to be mediated via changes in the amount of the enzyme protein rather than to the increased or decreased catalytic activity of the same amount of protein.

This conclusion is, however, only valid if the hypothetical less active form of ornithine decarboxylase possesses sufficient catalytic activity to metabolize the α-(difluoromethyl)ornithine to a reactive species. If, for example, the quiescent SV-3T3 cells contain a totally inactive form of the enzyme which is activated by serum, it would not be detected by this methodology. Therefore, parallel measurements were made by using a radioimmunoassay technique to quantitate the amount of reactive protein. The radioimmunoassay was set up by using mouse kidney ornithine decarboxylase as a standard, α-(difluoromethyl)[5-^{3}H]ornithine–ornithine decarboxylase conjugate as the tracer ligands, and a specific antiserum raised in rabbits against the mouse kidney enzyme (Seely & Pegg, 1983). A standard curve is shown in Figure 5. Samples containing 2–50 ng of enzyme protein could be assayed conveniently. The effect of serum stimulation on the amount of immunoreactive ornithine decarboxylase protein in the SV-3T3 cells is shown in Figure 6. This also varied in parallel to the enzymatic activity seen in Figure 1. These results, therefore, support the hypothesis that the changes in activity are brought about by modifications in the amount of enzyme protein.

Discussion

These results do not support the concept that ornithine decarboxylase activity in SV-3T3 cells is controlled by regu-

latory molecules or by posttranslational modifications. During the period after stimulation by serum ornithine decarboxylase, activity fluctuates over a 20-fold range, but there was less than 40% variation in the ratio of enzyme protein to activity whether the protein was measured by radioimmunoassay or by binding of α-(difluoromethyl)ornithine. This result is similar to that found in the mouse kidney after administration of androgens when a 400-fold rise in activity is paralleled by a rise in the amount of enzyme protein (Seely & Pegg, 1983). It is conceivable that the results in this tissue are a special case since the cells of the proximal tubules which contain most of the activity (Pegg et al., 1982a,b) are very much richer in ornithine decarboxylase than other mammalian cells. However, the present results provide evidence that ornithine decarboxylase activity in another, perhaps more typical, cell type is also regulated by changes in the amount of protein. Therefore, until positive evidence conclusively indicating the physiological relevance of the postulated mechanisms involving changes in the catalytic activity of a fixed amount of enzyme protein is provided, it is a reasonable hypothesis that the activity of this enzyme is regulated by changes in its rate of synthesis or degradation. It should be noted that this conclusion applies exclusively to mammalian cells in which the enzyme has a rapid rate of turnover (Russell, 1980; Pegg & Williams-Ashman, 1981; Seely et al., 1982a). In yeast, ornithine decarboxylase does not have a particularly short half-life, and it has been shown by immunological techniques that the loss of enzyme activity in response to exogenous polyamines is not accompanied by a decline in the protein (Tyagi et al., 1981, 1982). However, the mechanism by which inactivation occurs is not yet known. A similar situation may exist in the slime mold *Physarum polycephalum* where both conversion to a form having a low affinity for pyridoxal phosphate (Mitchell, 1981; Mitchell et al., 1982) and inactivation by phosphorylation (Atmar & Kuehn, 1981) have been observed.

Both of the methods used to determine the absolute amount of enzyme protein in the present study have some drawbacks and potential for error. However, most of these can be ruled out by appropriate controls, and the similarity of the results obtained by the two methods greatly strengthens the conclusions. It might be argued that the radioimmunoassay technique would not detect a modified form of the protein having less activity. However, the complex between ornithine decarboxylase and the macromolecular inhibitor termed antizyme (Canellakis et al., 1979) is precipitated by the antiserum as well as by the native enzyme itself (Seely & Pegg, 1983); aggregates of the enzyme of high molecular weight formed during prolonged storage are similar in immunoreactivity to the equivalent amount of native enzyme (unpublished results), and binding of inhibitors to the enzyme does not affect the radioimmunoassay (Seely & Pegg, 1983). Therefore, it is unlikely that a modification causing a complete loss of antigenicity toward the antiserum occurs. The technique for radioimmunoassay used here has the particular advantage that the α-(difluoromethyl)[5-^{3}H]ornithine–ornithine decarboxylase complex is used as the labeled antigen. The very high specificity of this drug for the protein avoids the possibility of labeling impurities in the preparation which frequently introduces artifacts into radioimmunoassays with iodinated antigens. The major disadvantages of the other technique used, the titration with α-(difluoromethyl)ornithine, are the somewhat limited sensitivity of the method and its inability to detect inactive forms of the enzyme. As indicated in Figure 2 and Table I, nonspecific binding becomes a substantial factor when samples containing less than 1 unit/mg of protein are exposed to the labeled drug, and samples containing substantially lower levels of activity must be partially purified to permit accurate determination of the specific binding. (It should be stressed that this conclusion in no way contradicts previous statements of the remarkable specificity of this drug. An enzyme activity of 1 unit/mg is equivalent to less than one part of enzyme protein in 1 000 000, and with this level, the nonspecific binding amounts to only about 30% of the total.)

The sensitivity of the titration is somewhat less than that of the radioimmunoassy which also can detect inactive forms. On the other hand, the titration with α-(difluoromethyl)[5-^{3}H]ornithine gives additional information in that the electrophoretic and chromatographic properties of the labeled protein can be determined. As shown in Figure 3, the subunit molecular weight of the ornithine decarboxylase in SV-3T3 cells at all times after stimulation was about 53 000, in good agreement with previous estimates of the molecular weight of the rat and mouse enzyme (Pritchard et al., 1981; Persson, 1981; Seely et al., 1982b; Kameji & Hayashi, 1982) and of the purified enzyme from SV-3T3 cells (Weiss et al., 1981). The enzyme was purified by Boucek and colleagues, but even after a 7000-fold purification, the specific activity of their purified enzyme was only 120 000 units/mg (Boucek & Lembach, 1977; Weiss et al., 1981) whereas our results suggest that the homogeneous enzyme would have a specific activity of about 1 300 000 units/mg. It is, therefore, probably not feasible to obtain sufficient quantities of the enzyme for structural studies from such cultured cells. However, overproducing cells selected for resistance to ornithine decarboxylase inhibitors (Steglich et al., 1982) and the mouse kidney enzyme (Persson, 1981; Seely et al., 1982b) may prove to be suitable sources.

Registry No. Ornithine decarboxylase, 9024-60-6.

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