Short Communications

Mammalian Ornithine Decarboxylase: Activation and Alteration of Physical Behaviour by Thiol Compounds

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(Received 13 July 1970)

Mammalian ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) is one of the enzymes that responds most rapidly and dramatically to various stimuli causing growth of tissues, such as in liver after partial hepatectomy (Jänne & Raina, 1968; Russell & Snyder, 1968; Fausto, 1969; Schrock, Oakman & Bucher, 1970) or growth hormone treatment (Jänne, Raina & Siimes, 1968), in the ventral prostate of castrated rats after administration of testosterone (Pegg, Lockwood & Williams-Ashman, 1970) or in cells grown in culture after stimulation by epidermal growth factor (Stastny & Cohen, 1970). In most instances large increases in ornithine decarboxylase activity occur within several hours after the appropriate treatment.

Ornithine decarboxylase from rat ventral prostate (Pegg & Williams-Ashman, 1968) and regenerating liver (Raina & Jänne, 1968) exhibits an absolute requirement for pyridoxal phosphate; there is no necessity for any specific metal ion. Raina & Jänne (1968) reported that ornithine decarboxylase obtained from regenerating rat liver by ammonium sulphate fractionation was activated markedly by 2-mercaptoethanol with a concomitant shift of the pH optimum to a more alkaline value. On the other hand, Schrock *et al.* (1970) stated that decarboxylation of ornithine by soluble ultracentrifuged extracts of regenerating rat liver was not enhanced by addition of 2-mercaptoethanol. Most studies in the literature on factors affecting ornithine decarboxylase in mammalian tissues have been carried out with assay systems to which no thiol compounds were added.

We have now found that the activity of prostatic and liver ornithine decarboxylase is stimulated intensely by dithiothreitol. In the absence of thiol compounds the enzyme undergoes remarkable changes in its physical behaviour.

Methods. Adult male Sprague-Dawley rats (300–350 g) were used. Partial hepatectomy was performed as described by Higgins & Anderson (1931). Tissue homogenates were centrifuged at 2°C for 20 min at 15 000g followed by centrifugation for 120 min at 45 000g. Ammonium sulphate

fractionation of the resultant supernatant fluid was performed at 0°C by addition of the solid salt. Ornithine decarboxylase activity was assayed as described by Raina & Jänne (1968) and Pegg & Williams-Ashman (1968); the composition of the reaction mixtures is shown in the protocols. Sucrose-density-gradient ultracentrifugations were carried out at 2°C in a Spinco L2-65B machine with an SW65 rotor. Gel filtrations were conducted on columns comprised of a lower layer (8.5 cm) of Bio-Gel P-100 and an upper layer (24 cm) of Bio-Gel P-200 (both from Bio-Rad Laboratories, Richmond, Calif., U.S.A.) by using an upflow technique with flow adaptors. The columns were equilibrated against 25 mM-tris-HCl (pH 7.5)–0.1 mM-EDTA in the absence or presence of 5 mM-dithiothreitol. The columns were calibrated with Dextran Blue 2000, potassium dichromate, yeast alcohol dehydrogenase and horse liver alcohol dehydrogenase (both enzymes from Sigma Chemical Co., St Louis, Mo., U.S.A.) for determinations of molecular Stokes radii by the method of Ackers & Steele (1967).

Results and discussion. Virtually all of the ornithine decarboxylase activity of soluble extracts of rat ventral prostate was precipitated by ammonium sulphate between 20% and 50% saturation [this is in contrast with the finding of Pegg & Williams-Ashman (1968) that most of the enzyme activity was precipitated by addition of neutralized solutions of ammonium sulphate to between 60% and 80% saturation, as opposed to use of the solid salt in the present experiments]. After dialysis, such preparations, even when fractionated in the presence of 10 mM-2-mercaptoethanol, were stimulated manyfold by addition of various thiol compounds to the assay system. A saturating concentration of 5 mM-dithiothreitol enhanced ornithine decarboxylase activity to about a fourfold greater extent than that found with a saturating concentration of 30 mM-2-mercaptoethanol. Considerably smaller increases in enzyme activity were evoked by 2,3-dimercaptopropanol, cysteine or GSH (none of these thiol compounds caused any non-enzymic

Fig. 1. Sucrose-density-gradient centrifugation and gel filtration of rat ventral-prostate ornithine decarboxylase present in material precipitated from soluble tissue extracts by $(\text{NH}_4)_2\text{SO}_4$ between 20% and 50% saturation. The latter material was dissolved in and dialysed against 25 mM-tris-HCl (pH 7.5)–0.1 mM-EDTA. Preparations were processed with (●; left-hand ordinates) or without (○; right-hand ordinates) 5 mM-dithiothreitol in all solutions. (a) A 0.2 ml portion (containing about 6 mg of protein) of the solution was overlaid on a linear 5–20% (w/v) sucrose gradient [in 25 mM-tris-HCl (pH 7.4)–0.1 mM-EDTA with or without 5 mM-dithiothreitol] and centrifuged at 2°C for 14 h at 60000 rev./min. Fractions of volume 0.2 ml were collected. The direction of sedimentation is from right to left. The arrow indicates banding of bovine serum albumin (BSA). (b) A 1 ml portion (about 30 mg of protein) of protein solution was applied to a Bio-Gel column (details under 'Methods') and eluted with 25 mM-tris-HCl (pH 7.5)–0.1 mM-EDTA with or without 5 mM-dithiothreitol. Fractions of volume 3 ml were collected. The arrow indicates the peak of the void volume (V_0). The incubation medium for assay of ornithine decarboxylase activity contained 50 μmol of glycylglycine buffer, pH 7.0, 0.2 μmol of L-ornithine, 0.4 μCi of DL-[1- ^{14}C]ornithine (containing 0.004 μmol of L-ornithine), 2.5 μmol of dithiothreitol, 0.1 μmol of pyridoxal 5-phosphate and the enzyme solution in a total volume of 0.5 ml. Radioactive CO_2 liberated was trapped into 0.2 ml of 1 M-Hyamine hydroxide. The reaction was allowed to proceed at 37°C for 60 min, and then halted with 1 ml of 40% (w/v) trichloroacetic acid and incubated in the closed system for another 20 min at 25°C so as to release all CO_2 dissolved in the reaction mixture. The recovery of total enzyme activity applied to both the sucrose density gradients (a) and the gel-filtration columns (b) was greater than 80% in every case.

decarboxylation of L-ornithine in the presence of pyridoxal phosphate).

When 5 mM-dithiothreitol was included in both the homogenization medium and in all solutions used for ammonium sulphate fractionation and dialysis, as well as in the reaction mixture used for assay of ornithine decarboxylase, the activity was observed to be almost twice that obtained when the preparations were processed in the presence of 10 mM-2-mercaptoethanol but assayed with 5 mM-dithiothreitol present, and fourfold greater than that of protein isolated in the absence of thiol compounds but again assayed in test systems containing 5 mM-dithiothreitol.

Fig. 1 indicates that addition of dithiothreitol to all solutions employed for enzyme isolation has a pronounced effect on the physical behaviour of prostatic ornithine decarboxylase. The protein precipitated by ammonium sulphate in the absence of ambient thiol compound exhibited less than one-quarter of the specific decarboxylase activity of material isolated in the presence of 5 mM-

dithiothreitol, yet much of the enzyme activity of the former preparations sedimented faster when subjected to ultracentrifugation through a linear 5–20% (w/v) sucrose density gradient (Fig. 1a). Similarly, in the absence of dithiothreitol, the enzyme activity was retarded to a smaller extent when subjected to gel filtration in comparison with preparations that were initially processed and eluted from the column with buffered solutions containing dithiothreitol (Fig. 1b). From the gel-filtration results it was reckoned that the molecular Stokes radius of the smaller form of the enzyme (obtained in the presence of dithiothreitol) was 3.87 nm and that that of the larger form was 4.52 nm. It seems possible that the larger and less active material represents a dimer of prostatic ornithine decarboxylase. The findings in Fig. 1(a) also hint that higher degrees of polymerization of the enzyme may occur in the absence of thiol compounds.

Comparable results were obtained in studies on the ornithine decarboxylase of normal and regenerating rat liver. The activity of fresh centri-

fused extracts from regenerating liver prepared in the absence of added thiols was hardly affected by addition of 5mM-dithiothreitol to the assay system. However, if such extracts were dialysed overnight at 2°C against 25mM-tris-HCl they lost about 80% of their ornithine decarboxylase activity as measured in the absence of 5mM-dithiothreitol, but showed no loss when assayed in the presence of this thiol. The degree of enhancement of ornithine decarboxylase at 22h after partial hepatectomy was considerably less if the tissue extracts were prepared as well as assayed in the presence of dithiothreitol, because the activity of normal liver preparations was increased to a relatively greater extent by the thiol compound. Omission of the thiol from preparative solutions led to changes in the sucrose-density-gradient centrifugation profiles of material from regenerating liver precipitated by ammonium sulphate between 20% and 50% saturation that were similar to those observed with the ventral-prostate preparations.

A very swift decline in the ornithine decarboxylase activity of crude soluble extracts of regenerating rat liver, prepared and tested with 5mM-dithiothreitol present, was observed over the first hour after injection of cycloheximide (15mg/kg body wt.) into animals that had been partially hepatectomized 22h previously. The observation corroborates the

view (Jänne & Raina, 1969; Russell & Snyder, 1969) that the half-life of hepatic ornithine decarboxylase is very short.

This research was supported in part by Research Grant HD-04592 from the U.S. Public Health Service.

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