Characterization of ornithine decarboxylase of tobacco cells and tomato ovaries

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Some characteristics of L-ornithine decarboxylase of tomato ovaries and tobacco cells are described. The enzyme has a pH optimum of 8.0. It requires pyridoxal phosphate and thiol reagent (dithiothreitol) for activity. It is specific for L-ornithine and has an apparent K_m of 1.4×10^{-4} M. It has an apparent molecular weight of 107000. Putrescine inhibited the activity *in vitro*. Spermidine and spermine also inhibit the enzyme, but less effectively. It is concluded that the enzyme is similar to that of mammalian origin and likewise fulfils a function related to cell proliferation.

L-Ornithine decarboxylase (EC 4.1.1.17) is the first enzyme controlling the rate-limiting step in the biosynthetic pathway of polyamines in mammalian cells. Ample information has been accumulated characterizing the mammalian enzyme and some of its regulatory properties (Canellakis *et al.*, 1979). Very little is known about the role of polyamines and their biosynthetic enzymes in cellular processes in plants (Smith, 1977).

We have demonstrated the apparent association of L-ornithine decarboxylase activity with rapidly proliferating plant cells (Heimer *et al.*, 1979). This finding differs from the commonly accepted belief (Smith, 1977, 1979; Yoshida, 1969; Bagni & Speranza, 1977; Montague *et al.*, 1978) that in plants putrescine is formed from L-arginine via agmatine by the enzyme L-arginine decarboxylase (EC 4.1.1.19).

The present paper describes some of the characteristics of L-ornithine decarboxylase from XD cells of tobacco in liquid suspension and from tomato ovaries 3–4 days after pollination.

Materials and methods

The XD line of tobacco cells (*Nicotiana tabacum* cv. *xanthi*) isolated by P. Filner were grown as previously described on nitrogen-free M-ID medium supplemented with casamino acids (Difco, Detroit, MI, U.S.A.) (1.5 g/litre) as a nitrogen source (Filner, 1966). Every 3 weeks the cells were subcultured. Tomato plants (*Lycopersicon esculentum* Mill line 367234) were grown in a glasshouse under natural light conditions. Plants were trained to one stem.

Flowers were hand pollinated at full anthesis and tagged.

L-Ornithine decarboxylase was extracted (at 4°C) and assayed (at 25°C) as previously described (Bachrach, 1976), with some modifications. Tris/HCl buffer (pH 8.0) was used at concentrations of 0.25 M and 0.1 M for the extraction of tomato tissues and tobacco cells respectively. The concentration of dithiothreitol was increased to 10 mM. Tobacco cells were homogenized in a Teflon/glass homogenizer. Tomato tissues were pulverized with a mortar and pestle. The homogenate was clarified by centrifugation at 10000 *g* for 20 min. The supernatant was fractionated with $(\text{NH}_4)_2\text{SO}_4$. The fraction precipitated between 0 and 50% saturation of $(\text{NH}_4)_2\text{SO}_4$ was collected, redissolved in the extraction buffer to its original volume and dialysed for 19 h against 0.025 M-Tris/HCl buffer (pH 8.0) containing 1 mM-dithiothreitol, 50 μM -EDTA and 25 μM -pyridoxal phosphate, designated 'complete dialysis solution'. Gel filtration was performed in a column of Sephadex G-200 equilibrated with the same buffer used for dialysis. Sample volume was 0.5 ml, and fraction volume 0.75 ml. Flow rate was 5.0 ml/h.

For the enzyme assay, 0.5 ml of enzyme (equivalent to 125 mg fresh wt.) was incubated with 0.1 ml of L-[1- ^{14}C]ornithine (sp. radioactivity 59 Ci/mol). The radioactivity of the substrate was 5 $\mu\text{Ci/ml}$.

The stoichiometry of the decarboxylation of L-ornithine and the formation of putrescine was investigated with L-[1- ^{14}C]ornithine and DL-[5- ^{14}C]ornithine as the substrates. Putrescine was separated from ornithine by one of two separation systems: (a) the dansylated (5-dimethylaminonaphthalene-1-

sulphonylated) compounds in the reaction mixture were separated on a silica-gel G t.l.c. plate with ethyl acetate/cyclohexane (2:3, v/v) as the solvent; (b) the hydrochloride derivatives of the compounds in the reaction mixture were separated on Whatman no. 1 paper by descending chromatography with methanol/pyridine/water/acetic acid (6:6:4:1, by vol.).

Results and discussion

In studies on animal L-ornithine decarboxylase 0.025 M-Tris/HCl buffer containing 2.5 mM-dithiothreitol is used. The occurrence of polyphenols and their oxidases in plant tissues made it necessary to increase the ionic strength of the extraction buffer to 0.1 M- and 0.25 M-Tris/HCl for tobacco cells and tomato ovaries, respectively, and the concentration of dithiothreitol to 10 mM. Under these conditions the recovery of enzyme activity was maximal. After extensive dialysis, the enzyme from both sources could be kept at -20°C without apparent loss of activity. However, repeated freezing and thawing caused loss of activity, especially of the tomato enzyme. The optimal pH for the activity of the tobacco enzyme is 8.0 (Fig. 1), which is somewhat different from that of the mammalian enzyme, i.e. pH 7.1 (Bachrach, 1976). The enzyme requires pyridoxal phosphate and a thiol reagent (Table 1). Omitting either of them from the dialysis buffer caused a drastic loss of enzyme activity. The activity could not be restored by supplementing the assay mixture with the missing factors (Table 1).

The requirement of the enzyme for pyridoxal phosphate can be further demonstrated by testing the effect of L-canaline on the enzyme (Fig. 2). This non-protein amino acid was shown to inhibit various pyridoxal phosphate-dependent enzymes from

animal sources. It was suggested (Rahiala *et al.*, 1971) that the binding of L-canaline to pyridoxal phosphate (free and enzyme-bound) is the cause of the inhibition. Indeed, the L-ornithine decarboxylase derived from tobacco cells is inhibited by L-canaline (Fig. 2).

Fig. 1. Dependence of tobacco L-ornithine decarboxylase activity on the pH of the assay mixture. pH range 6–8 was obtained with 0.1 M-phosphate buffer (●) and pH range 7–9 with 0.1 M-Tris/HCl buffer (■). In these assays 0.5 ml of enzyme was mixed with 0.25 ml of substrate.

Table 1. Requirement for pyridoxal phosphate (PLP) and a thiol reagent, dithiothreitol (DTT), by L-ornithine decarboxylase from tobacco cells

The partially purified enzyme was dialysed overnight against the indicated buffers. Enzyme activity in the dialysed extract was determined in the presence or absence of the indicated compounds in the reaction mixture. Abbreviations: nd, not detectable.

Expt. no.	Dialysis buffer	Assay buffer	L-Ornithine decarboxylase activity	
			(nmol/h)	(% of control)
I	Tris/HCl + DTT + PLP	Tris/HCl + DTT + PLP	0.597	100
	Tris/HCl + PLP	Tris/HCl + PLP	0.193	32
	Tris/HCl + DTT	Tris/HCl + DTT	0.065	11
	Tris/HCl	Tris/HCl	0.035	6
II	Tris/HCl + DTT + PLP	Tris/HCl + DTT + PLP	1.540	100
	Tris/HCl	Tris/HCl	nd	—
	Tris/HCl	Tris/HCl + DTT	0.013	1
	Tris/HCl	Tris/HCl + PLP	nd	—
	Tris/HCl	Tris/HCl + DTT + PLP	0.014	1

Fig. 2. Effect of increasing concentrations of L-canaline on the activity of tobacco L-ornithine decarboxylase. L-Canaline was added to the assay mixture at the indicated final concentrations. The arrow indicates the concentration of pyridoxal phosphate in the assay mixture; 100% activity represents 10.323 nmol/h per g fresh wt. L-Canaline did not affect enzyme activity at 0.1 μM.

The decarboxylation of L-ornithine was not the result of a non-specific decarboxylating activity present in the extract, since isotope dilution of $^{14}\text{CO}_2$ derived from L-[1- ^{14}C]ornithine could be obtained only by non-labelled L-ornithine (Tables 2 and 3). Neither L-arginine nor a mixture of amino acids had any effect (Tables 2 and 3). The yield of putrescine from L-ornithine was 90% of the theoretical amount. Furthermore, no liberation of $^{14}\text{CO}_2$ from L-[5- ^{14}C]ornithine was observed (results not shown). This result indicates that the liberation of $^{14}\text{CO}_2$ from labelled L-ornithine could not be due to oxidative or other type of breakdown of the entire L-ornithine molecule.

Putrescine, the end product of the decarboxylation, inhibited L-ornithine decarboxylase *in vitro*, whereas spermidine and spermine, higher-molecular-weight polyamines, were less effective (Table 4). This fact points to the possible end-product regulation of the enzyme activity.

The K_m of partially purified L-ornithine decarboxylase was $1.4 \times 10^{-4}\text{M}$. By the use of a calibrated Sephadex G-200 column, an apparent molecular weight of 107 000 was determined. The molecular weight of the animal L-ornithine decarboxylase was

Table 2. Effect of ornithine and casamino acids added to the assay mixture on the release of $^{14}\text{CO}_2$ from L-[1- ^{14}C]ornithine by L-ornithine decarboxylase from tobacco cells. For details see the text.

Expt. no.	Amino acid	$^{14}\text{CO}_2$ released from L-[1- ^{14}C]ornithine	
		(c.p.m.)	(% of control)
I	None*	54 000	100
	L-Ornithine (0.16 mM)	27 750	47
	L-Ornithine (1.6 mM)	5000	9
II	None†	79 000	100
	Casamino acids (16 mM)	76 000	96

* The concentration of L-[1- ^{14}C]ornithine in the assay mixture was 16 μM.

† The molarity of casamino acids was calculated by assuming the average molecular weight of amino acids to be 100 and the occurrence of 50% (w/w) salt in the casamino acids preparation.

Table 3. Effect of ornithine, arginine and amino acid mixture on the release of $^{14}\text{CO}_2$ from L-[1- ^{14}C]ornithine by L-ornithine decarboxylase from tomato fruits. For details see the text. The amino acid mixture contained the following, each at 0.16 mM: L-aspartic acid, L-phenylalanine, L-methionine, L-histidine, L-lysine, L-alanine, L-isoleucine, L-valine, L-leucine, L-serine, glycine, L-tyrosine, L-glutamic acid, L-arginine.

Expt. no.	Treatment	$^{14}\text{CO}_2$ released from L-[1- ^{14}C]ornithine	
		(c.p.m.)	(% of control)
I	(1) Control (complete medium)	153 200	100
	(2) +L-Ornithine (0.16 mM)	50 760	33.0
	(3) +L-Arginine (0.16 mM)	138 600	90.4
	(4) +L-Ornithine (0.16 mM)	54 680	35.7
	+ L-arginine (0.16 mM)		
II	(1) Control	188 950	100
	(2) +L-Ornithine (0.16 mM)	61 680	33
	(3) +Amino acid mixture	198 960	105
	(4) +L-Ornithine (0.16 mM)	65 590	35
	+ amino acid mixture		

Table 4. *Effect of various polyamines on the activity of tobacco L-ornithine decarboxylase in vitro*

In this experiment 0.25 ml of enzyme was mixed with 0.05 ml of substrate. For other details see the text.

Polyamine added to assay mixture	Ornithine decarboxylase activity	
	(nmol/h)	(% of control)
None	0.210	100
Putrescine (0.16 mM)	0.038	18
Spermine (0.16 mM)	0.139	66
Spermidine (0.16 mM)	0.129	61

reported to be 100 000 (Obenrader & Prouty, 1977).

The characteristics of the plant L-ornithine decarboxylase, namely substrate specificity, requirement for cofactors, K_m , optimal pH, molecular weight and close association with mitotic activity (Heimer *et al.*, 1979) suggest that the enzyme fulfils a function similar to that of the mammalian enzyme. It is possible, however, that the increase in the enzyme activity on subculturing of XD cells or the pollination of tomato ovaries only reflects a change in the biochemical steady state of the cells. However, L-ornithine decarboxylase activity is relatively low in mature fully expanded leaves, having metabolically active but mitotically less active cells, as well as in XD cells in stationary phase (Heimer *et al.*, 1979).

We propose that L-ornithine decarboxylase of tomato and tobacco cells does indeed fulfil a function related to cell proliferation similar to that of mammalian cells.

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