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ORNITHINE DECARBOXYLASE FROM EMBRYOS OF JUTE SEEDS

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Key Word Index—*Corchorus olitorius*; Tiliaceae; jute; ornithine decarboxylase; polyamines; putrescine.

Abstract—After purification from jute embryos ornithine decarboxylase (EC 4.1.1.17) was separated into two fractions by gel chromatography. Fraction-A was purified to homogeneity and characterized. It had an apparent M_r of 39000 and showed maximum activity at pH 7.8. The enzyme was specific for L-ornithine and followed Michaelis–Menten kinetics with K_m of 10^{-4}M. Pyriodoxal phosphate acts as a cofactor of the enzyme.

INTRODUCTION

In animal cells putrescine is formed from L-ornithine by ornithine decarboxylase (ODC) but in plants there are two alternative pathways; putrescine may be formed from arginine via agmatine by arginine decarboxylase (ADC) or directly from ornithine by ODC. Recently, ODC and ADC activities have been detected in tobacco and tomato cells indicating that in higher plants arginine and ornithine may serve as precursors for putrescine biosynthesis [1, 2]. ODC activity has also been observed in *Phaseolus vulgaris* and oat and sunflower [3–5]. In young developing tomato fruit, ODC and not ADC is the main enzyme for putrescine biosynthesis [6]. It has been proposed that ODC may be active during cell proliferation and ADC is required for growth by expansion and differentiation [7]. Although ADC has been purified from various sources [8–13], ODC has only been partially purified from germinated barley seeds [14, 15]. We have now characterized this enzyme from jute seeds, where it is present in an appreciable amount during early germination.

RESULTS AND DISCUSSION

With the procedure described in the Experimental, 773-fold purification of the enzyme (fraction-A) with a recovery of 33% was achieved by means of molecular exclusion chromatography. Table 1 summarizes the purification steps of protein preparation. The purity of the enzyme was judged by a sharp single band of protein in polyacrylamide gel electrophoresis under non denaturing conditions.

Tabor and Tabor have purified ODC to homogeneity from *S. cerevisiae*. They showed a single band, indicating the monomeric nature of the enzyme [16]. Mitchell and Carter observed two forms of ODC (A and B) in *P. polycephalum* during purification which differ in their affinity for pyridoxal phosphate [17]. Seely and Pegg have also separated two fractions of ODC from mouse kidney [18].

Optimum conditions for enzyme assay

The enzyme exhibited a narrow range of pH activity with a maximum at pH 7.8 in Tris–HCl buffer with ornithine as substrate. It shows 50% of activity near pH 7.4. ODC activity was measured at various temperatures ranging from 20–50°. At pH 7.8, ODC was most active at 37°.

The M_r of the enzyme determined by Sephadex G-100 gel filtration was estimated to be 39000 whereas the M_r of ODC from *P. Polycephalum* [19] and Calf liver [20] are 43000 and 54000 respectively. Pyridoxal phosphate (PLP) at 20 μM stimulated the enzyme activity by 20–25%. The maximum enzyme activity was obtained in the presence of 5 mM DTT. The requirement of thiol compounds for enzyme activity has also been reported in

Table 1. Purification of ornithine decarboxylase from *Corchorus olitorius*

	Steps	Total protein (mg)	Specific activity protein (dpm/mg)	Purification (fold)	Recovery (%)
1.	Crude extract	465	140	1	100
2.	$(NH_4)_2SO_4$ ppt.	100	267	2	41
3.	DEAE-Cellulose	1.5	90000	639	75
4.	Sephadex G-100 (Fraction A)	0.2	108000	773	33

Table 2. Effect of Inhibitors on ornithine decarboxylase

Name of inhibitors	Concentration (mM)	% of inhibition
Putrescine	10	62
Cadaverine	1	62
	10	80
Spermine	1	48
	10	75
Spermidine	10	50
DFMO	10	0

mammalian systems [21]. It exhibited typical Michaelis–Menten kinetics with a K_m of 10^{-4}M. Heimer and Mizrahi have reported an apparent K_m of 1.4×10^{-4} M for ODC from tobacco and tomato ovaries [22]. Kyriakidis and his co-workers reported K_m of 10^{-3} M from germinated barley seeds [15]. The enzyme was stable for 15 days at 4° but activity was completely lost by boiling for 10 min.

Effect of inhibitors

Polyamines are potent inhibitors of ODC [16]. In the case of putrescine and spermidine, more than 1 mM is required for inhibition of the enzyme whereas spermine and cadaverine at 1 mM inhibited the enzyme by 48% and 62%. EDTA inhibits the enzyme activity slightly. However DFMO, a suicide inhibitor of ODC, does not inhibit the enzyme *in vitro* even at 1–10 mM concentration. PMSF at 5 mM suppressed the enzyme activity by 60%.

EXPERIMENTAL

Chemicals. DEAE cellulose, Sephadex G-100 were from Sigma, L-[1-^{14}C] ornithine hydrochloride (specific activity 57 mCi/mmol) were from Amersham U.K.

Material. Jute seeds were obtained from Jute Research Institute in Barrackpur, West Bengal. After sterilization in 0.1% Hg_2Cl_2, the seed was germinated on moistened filter paper in Petri dishes at $37 \pm 1°$ for 16 hr.

The enzyme activity was measured following the method of ref. [23] with slight modifications. The assay mixture in a total vol. of 1 ml contained 40 mM Tris–HCl buffer pH 7.8 (0.5–2.0) mg of enzyme, 20 μM PLP, 5 mM DTT and 0.1 μCi L-(1 – ^{14}C) ornithine was incubated at 37° for 1 hr. The reaction was terminated by addition of 0.5 ml of 4 N H_2SO_4. The released $^{14}CO_2$ was trapped with 0.5 ml of M KOH in the centre tube. Protein was determined by the method of ref. [24].

Purification. Step 1:16 hr germinated jute embryos were homogenized in Tris–HCl buffer (50 mM, pH 7.6). The homogenate was filtered through cheese cloth centrifuged at 26 000 g for 20 min. Step 2: The crude extract was subjected to $(NH_4)_2SO_4$ fractionation and the protein pptd at 30–60% satn was dispersed in Tris–HCl buffer (50 mM, pH 7.6) and dialysed against the same buffer for 24 hr. Step 3: The dialysed fraction was applied to a DEAE cellulose column (14 × 2 cm) previously equilibrated with Tris–HCl buffer. The enzyme fractions were eluted with the following linear gradient: 200–300 mM KCl in 50 mM Tris–HCl buffer. The active fractions were pooled and concd by 70% $(NH_4)_2SO_4$ pptn. After centrifugation at 26 000 g for 20 min, the ppt. was dissolved in 50 mM Tris–HCl buffer and dialysed overnight against the same buffer. Step 4:The dialysed fraction obtained in step 3 was passed through Sephadex G-100 column (23 × 2 cm) equilibrated with 50 mM Tris–HCl buffer (pH 7.6). With $V_o = 22$ ml, $V_t = 68$ ml, fraction A and B were eluted after 50 and 130 ml respectively. The specific activity of fraction A is greater than that of fraction B.

Polyacrylamide gel electrophoresis This was carried out in 7.5% gel at pH 8.5 using Tris–glycine buffer according to the method of ref. [25]. Gels were stained with Coomassie Brilliant Blue and destained with 7.5% HOAc.

The M_r of the enzyme was determined by Sephadex G-100 column using as reference proteins chymotrypsinogen A (25 000), ovalbumin (45 000), bovine serum albumin (68 500), hexokinase (96 500), cyt *c* (12 400) in presence of Pi buffer (0.1 M, pH 7.0) containing 0.2 M NaCl.

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