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SHORT COMMUNICATION

ARGINASE FROM *LATHYRUS SATIVUS*

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Abstract—The seeds of *Lathyrus sativus* contain the unusual amino acid homoarginine. The possible breakdown of homoarginine to lysine and urea has been investigated with enzyme extracts prepared from the seedlings of *L. sativus*. The results indicate that there is no separate homoarginase enzyme but that the arginase present has about 5 per cent activity towards L-homoarginine as compared to that obtained with L-arginine. The enzyme does not show an absolute dependence on Mn^{2+} for activity and maximal activation of the enzyme has been realized with Fe^{3+}. It is concluded that the breakdown of homoarginine through the urea cycle may only represent a minor pathway for the catabolism of this compound in this plant.

INTRODUCTION

Lathyrus species contain unusual amino acids such as homoarginine[1,2] and oxalyl amino acids.[3,4] Homoarginine has been found to give rise to lathyrine in *L. tingitanus* through a series of conversions involving γ-hydroxyhomoarginine and its lactone.[5] However, *L. sativus* contains only L-homoarginine and neither lathyrine nor the hydroxy derivatives of homoarginine can be detected in the seeds. So, the possible breakdown of homoarginine to urea and lysine by enzyme extracts prepared from the seedlings of *L. sativus* has been investigated.

RESULTS AND DISCUSSION

The partially purified enzyme preparation has 5 per cent activity towards homoarginine as compared to that obtained with arginine. Lysine has been identified as the other product of the reaction by its paper and column chromatographic behaviour. The ratio of activity towards arginine and homoarginine remains constant through the steps of purification (Table 1). The K_m values of the enzyme for arginine and homoarginine are $5{\cdot}5 \times 10^{-3}$ M and $1{\cdot}6 \times 10^{-2}$ M respectively. The pH optimum for the enzyme is between 9·5–10·0 with respect to both substrates. Table 2 shows some of the other properties of the enzyme. The enzyme when dialysed against EDTA (10^{-3} M) for 8 hr retains appreciable activity. Addition of manganese or cobalt to such an enzyme preparation increases the activity only by 23 per cent; however, addition of Fe^{3+} results in nearly a two-fold increase in activity. Arginase from several other sources is known to exhibit a striking dependence on Mn^{2+} and Co^{2+} for activity.[6,7] Metal ions have also been known to effect changes in the pH-activity curve of

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arginase from jackbean[8] and liver.[9] However, such effects have not been noticed with the *Lathyrus sativus* enzyme. L-Ornithine and L-lysine inhibit this enzyme activity towards arginine as well as homoarginine. High concentrations of homoarginine at low enzyme

TABLE 1. PARTIAL PURIFICATION OF ARGINASE FROM *L. sativus*

Purification step	Urea formed μmoles/mg protein: Arginine A	Urea formed μmoles/mg protein: Homoarginine B	Ratio A/B
Crude	9·1	0·5	18·2
$(NH_4)_2SO_4$ (0–60 fraction)	22·5	1·1	20·5
$(NH_4)_2SO_4$ (35–55 fraction)	55·0	2·8	19·7

The assay mixture in 2 ml volume contained: L-arginine or L-homoarginine, 20 μmoles; $MnSO_4.H_2O$, 12 μmoles; glycine–NaOH buffer (pH 9·6), 100 μmoles. Usually 100 μg and 2 mg protein were added for the arginine and homoarginine systems respectively. The reaction was stopped with trichloroacetic acid after 45 min incubation at 37° and aliquots were taken for urea estimation.

TABLE 2. PROPERTIES OF ARGINASE FROM *L. sativus*

Reaction condition	Urea formed μmoles/mg protein: Arginine	Urea formed μmoles/mg protein: Homoarginine
Undialysed enzyme (complete)	63·5	3·0
Dialysed enzyme (metal omitted)	53·9	2·40
$MnSO_4.H_2O$ ($6·5 \times 10^{-3}$ M)	66·7	3·1
$CoCl_2.6H_2O$ ($6·5 \times 10^{-3}$ M)	66·7	3·3
Ferric citrate ($6·5 \times 10^{-3}$ M)	104·2	4·5
L-Ornithine ($1·1 \times 10^{-3}$ M)	50·1	2·0
L-Ornithine ($5·4 \times 10^{-3}$ M)	21·2	1·1
L-Lysine ($1·1 \times 10^{-3}$ M)	54·6	2·5
L-Lysine ($5·4 \times 10^{-3}$ M)	25·5	1·0
L-Homoarginine ($4·0 \times 10^{-2}$ M)	57·0	—

The enzyme, prepared as described in the Experimental, was dialysed for 8 hr against glycine–NaOH buffer (pH 9·6, 0·05 M) containing 10^{-3} M EDTA with five changes of buffer. The metal ion effects were studied on the dialysed enzyme. The effects of L-ornithine, L-lysine and L-homoarginine were studied with the undialysed enzyme with Mn^{2+} as the activating metal ion. The enzyme assay details are described in Table 1.

concentrations slightly inhibit the activity towards arginine. All these data indicate that *L. sativus* does not contain a separate homoarginase but that the arginase present has a low affinity for homoarginine. Greenstein *et al.*[9] could detect hydrolysis of homoarginine by

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rat-liver arginase only after 12 hr, but not after 30 min, incubation. Brown[6] has reported that avian liver arginase has no activity towards L-homoarginine. However, a recent report[10] indicates that bovine liver arginase (Sigma preparation) has nearly 5 per cent activity towards L-homoarginine. The catabolism of L-homoarginine through the urea cycle may only represent a minor pathway for the degradation of this guanido amino acid. No evidence can be found for the participation of homoarginine in transamidinase systems from *L. sativus* although a corresponding reaction for arginine can be detected.

EXPERIMENTAL

Lathyrus sativus seeds were germinated for 96 hr and then extracted with glycine–NaOH buffer (pH 9·6, 0·25 M). The crude extract was centrifuged and the nucleoproteins were removed by manganous sulphate precipitation. The supernatant was subjected to $(NH_4)_2SO_4$ fractionation. The 0–60 $(NH_4)_2SO_4$ fraction was desalted on a Sephadex G-25 (coarse) column and again precipitated with $(NH_4)_2SO_4$. The 35–55 fraction was collected, dialysed for 60 min and used as the enzyme source. Urea was estimated using isonitroso propiophenone reagent.[11] There is no urease activity in the enzyme preparations under the conditions employed for arginase assay.

[10] W. L. RYAN, A. J. BARAK and R. J. JOHNSON, *Arch. Biochem. Biophys.* **123**, 294 (1968).
[11] E. L. OGINSKY, in *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN), Vol. 3, p. 639, Academic Press, New York (1957).