Phytochemistry, Vol. 21, No. 9, pp. 2201–2203, 1982.
Printed in Great Britain.

0031-9422/82/092201-03$03.00/0

PURIFICATION AND PROPERTIES OF *N*-CARBAMYL-PUTRESCINE AMIDOHYDROLASE FROM MAIZE SHOOTS

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(*Revised received* 8 *February* 1982)

Key Word Index—*Zea mays*; Gramineae; maize; *N*-carbamylputrescine amidohydrolase.

Abstract—*N*-Carbamylputrescine (NCP) amidohydrolase was purified *ca* 70-fold from maize shoots. The enzyme was present in the cytosol and the optimal pH was 6.5–7.0. The enzyme had a high substrate specificity and the K_m for NCP was 9×10^{-5} M. The energy of activation was 11.7 kcal/mol. The MW of the enzyme estimated by gel filtration was 125000. Cu^{2+}, Zn^{2+} and *p*-hydroxymercuribenzoate (*p*-HMB) were inhibitors of the enzyme.

INTRODUCTION

N-Carbamylputrescine (NCP) is an intermediate in putrescine biosynthesis in some higher plants [1, 2]. It is known that NCP is converted to putrescine by two mechanisms: (a) NCP amidohydrolase found in some higher plants which hydrolyses NCP to putrescine, carbon dioxide and ammonia [3]; and (b) putrescine carbamoyltransferase found in *Streptococcus faecalis* which reversibly produces putrescine and carbamoylphosphate from NCP and inorganic phosphate [4, 5]. In addition, putrescine synthase which has activities of agmatine iminohydrolase, putrescine carbamoyltransferase, ornithine carbamoyltransferase and carbamate kinase in a single polypeptide, has been purified recently from *Lathyrus sativus* seedlings [6].

Smith [3] has reported some properties of NCP amidohydrolase from barley but this enzyme has not been purified. In this paper, we describe the partial purification and some properties of NCP amidohydrolase (*N*-carbamoylputrescine N^5-carbamoyldihydrolase; EC 3.5.1.–) from maize shoots.

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RESULTS AND DISCUSSION

Purification of NCP amidohydrolase

NCP amidohydrolase was purified *ca* 70-fold from the extract of maize shoots with ammonium sulphate precipitation, acid treatment, ultracentrifugation and Sephadex G-150 gel filtration. The results are summarized in Table 1. Further purification with DEAE-cellulose or DEAE-Sephacel or NCP-affinity chromatography was unsuccessful in terms of yield and purification factor.

Subcellular distribution

Enzyme activity was found only in the 100000 *g* supernatant (4.3 pkat/mg protein) on differential centrifugation. This result agrees with the finding of Smith for the barley enzyme [3].

Effect of pH and temperature

The optimal pH of the maize enzyme was 6.5–7.0; in comparison the optimal pH for the barley enzyme

Table 1. Purification of maize NCP amidohydrolase

Step	Fraction	Total protein (mg)	Total activity (nkat)	Specific activity (pkat/mg)	Purification (fold)	Yield (%)
1.	Crude extract	2350	5.97	2.5	1	100
2.	40–65% ammonium sulphate ppt	470	4.38	9.3	3.7	73.4
3.	Sephadex G-25 after acid treatment	194	4.17	21.5	8.6	69.9
4.	Sephadex G-150	10.5	1.86	177	70.8	31.2

was 7.0–8.0 [3]. The energy of activation was 11.7 kcal/mol from the activity determined at 15–45°. The value for the barley enzyme was 13.5 kcal/mol [3]. The enzyme lost 47% of the activity on exposure at 50° for 10 min. Storage at 4° for 3 days resulted in 40% loss of activity.

Stoichiometry and substrate specificity

The stoichiometry of the enzyme reaction was obtained by measuring the disappearance of NCP and appearance of carbon dioxide and ammonia. Duplicate experiments showed that 1 mol of NCP was converted to 1 mol each of putrescine, carbon dioxide and ammonia. NCP amidohydrolase had a high substrate specificity, and a Lineweaver–Burk plot gave a K_m of 9×10^{-5} M. No detectable activity was found with carbamyl β-alanine, carbamyl aspartic acid, citrulline, urea, asparagine and glutamine (each at 7.5 mM) as substrates.

The MW of the enzyme

The apparent MW of the enzyme estimated by Sephadex G-200 gel filtration was 125000. Putrescine carbamoyltransferase of *Streptococcus faecalis* was a dimer of MW 140000 [5].

Inhibition

Enzyme activity was inhibited 88 and 53% by *p*-HMB (0.5 mM) and iodoacetamide (1 mM) respectively, on 10 min pre-incubation at 20°. The *p*-HMB-inhibited enzyme was partially reactivated by adding reduced glutathione (10-fold concentration). These results suggest that maize NCP amidohydrolase as well as barely enzyme [3] are sulfhydryl enzymes. The enzyme was also inhibited 96 and 98% by Cu^{2+} and Zn^{2+} (each at 1 mM) respectively, but not by Mg^{2+}, Co^{2+}, Mn^{2+} and Fe^{3+} (each at 1 mM). The inhibition by Cu^{2+} and Zn^{2+} may be due to their reaction with sulfhydryl group(s) in the enzyme. On the other hand, EDTA (10 mM), NaF, semicarbazide and acetohydroxamic acid (each at 1 mM) had no effect. Arginine, citrulline, ornithine, urea, agmatine, putrescine, spermidine, spermine, β-alanine and γ-aminobutyric acid were also without effect.

Related enzyme system

Although ornithine carbamoyltransferase of pea seedlings causes putrescine carbamoylation with the formation of NCP [7], a similar reaction was not observed in maize shoots.

EXPERIMENTAL

Plant materials. Maize seeds (*Zea mays* L. cv Goldencross Bantam T51) were germinated in moist Vermiculite at 25° for 2 days in the dark. Seedlings were transferred to plastic trays containing Hoagland soln and they were grown under continuous light (*ca* 3 klx at plant level) at 25° for 7–10 days. The excised shoots were sterilized with benzalkonium chloride, and thoroughly washed with deionized H_2O.

Purification. The shoots (400 g) were homogenized in a chilled Waring blender with 4 g polyvinylpyrrolidone (PVP) and 800 ml soln A [50 mM Tris–HCl buffer (pH 7) containing 5 mM mercaptoethanol, 0.1 mM EDTA and 20% glycerine]. The homogenate was passed through several layers of cheese cloth. After centrifugation (10000 *g*, 15 min), the supernatant was fractionated with $(NH_4)_2SO_4$ (40–65% satn). The ppt was collected by centrifugation (10000 *g*, 15 min) and dissolved in 100 ml soln A. The enzyme soln was adjusted to pH 4.5 with 10% HOAc and then centrifuged (10000 *g*, 15 min). The supernatant was adjusted to pH 7 with 10% NaOH and brought to 70% satn with solid $(NH_4)_2SO_4$. After centrifugation (10000 *g*, 15 min), the ppt was dissolved in a minimal amount of soln B (soln A without glycerine) and desalted on Sephadex G-25 equilibrated with soln B. The void fraction of eluate was ultracentrifuged (100000 *g*, 1 hr) and the supernatant was concd in a cellophane tube in contact with solid polyethylene glycol (MW-20000). After centrifugation (10000 *g*, 15 min), the concd enzyme soln (7 ml) was applied to a Sephadex G-150 column (3.2 × 90 cm) equilibrated with soln B (15 ml/hr). The active fractions were pooled.

Enzyme activity was determined by measuring the NH_3 released from NCP in Conway microdiffusion units [8]. NCP was prepared by the method of ref. [1] and recrystallized from EtOH. The reaction mixture contained 100 μmol Tris–HCl buffer (pH 7), 1 mg each of streptomycin sulphate and chloramphenicol, 15 μmol NCP and enzyme soln in a total vol. of 2 ml. After incubation for 24 hr at 30°, the reaction was terminated by adding 0.1 ml 50% TCA. The liberated NH_3 was determined with Nessler's reagent [9]. Protein was determined by the method of ref. [10] with BSA as a standard.

Subcellular distribution. Shoots (10 g) were homogenized in a chilled mortar with 1 g PVP and 20 ml 50 mM Tris–HCl buffer (pH 7) containing 5 mM mercaptoethanol, 0.1 mM EDTA and 0.45 M sucrose. The homogenate was passed through several layers of cheese cloth and subjected to differential centrifugation.

Stoichiometry studies. The enzyme from step 4 (Table 1) (2.5 mg protein) was used and the reaction mixture was the same as that of enzyme assay conditions with 7.5 μmol of NCP. Incubation was for 5 hr, and CO_2 was estimated manometrically. NCP and putrescine were separated by HVE on paper (3000 V, 15 min, 1 M HOAc) and estimated colorimetrically [11, 12].

The MW of the enzyme was estimated by gel filtration using Sephadex G-200 according to the method of ref. [13].

Acknowledgement—This work was supported in part by a Grant-in-Aid for Scientific Research (154227) from the Ministry of Education, Science and Culture, Japan.

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