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PURIFICATION AND PROPERTIES OF AGMATINE IMINOHYDROLASE FROM GROUNDNUT COTYLEDONS

R. K. SINDHU* and H. V. DESAI

Biochemistry Department, M.S. University, Baroda, India

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Key Word Index—*Arachis hypogea*; Leguminosae; groundnut; agmatine iminohydrolase; purification; kinetics; polyamines.

Abstract—Agmatine iminohydrolase was purified *ca* 375-fold from groundnut cotyledons. The enzyme exhibited an optimum pH between 5.5 and 8.5 and the energy of activation was 22 kcal/mol. The K_m for agmatine was (7.57 ± 0.77) × 10^{-4} M. The enzyme was inhibited by tryptamine, putrescine, cadaverine, spermidine and spermine. Inhibition by cadaverine and spermidine was competitive. The K_i values for cadaverine and spermidine were 4.1 × 10^{-3} and 7.5 × 10^{-4} M, respectively.

INTRODUCTION

The diamine, putrescine, occupies a central role in the biosynthetic pathways of polyamines since it serves as the obligatory precursor of spermidine and spermine in various organisms hitherto examined [1, 2]. In *Escherichia coli*, two pathways of putrescine formation co-exist, one from ornithine by direct decarboxylation as in animals and the other from arginine through agmatine; the relative extents of the two operations are determined by culture conditions [3]. However, in higher plants, putrescine biosynthesis appears to proceed either by the decarboxylation of arginine to agmatine (and its subsequent conversion to putrescine [2]) or via ornithine which can be decarboxylated directly to putrescine [4, 5]. The putrescine formed by either pathway may be converted to the polyamines spermidine and spermine [2]. Agmatine iminohydrolase, which converts agmatine to *N*-carbamylputrescine and ammonia, has been reported to be present in the leaves and seeds of maize and sunflower seedlings [6]. Attempts by Smith [6] to purify this enzyme were unsuccessful and only an enrichment of 2–5-fold could be achieved. In this paper, we report the purification and properties of agmatine iminohydrolase from groundnut cotyledons.

* Present address: Biochemistry Department, Postgraduate Institute of Medical Education and Research, Chandigarh-160012, India.

RESULTS AND DISCUSSION

Purification

Agmatine iminohydrolase has been purified *ca* 375-fold (Table 1) from the cotyledons of groundnut seedlings. The enzyme activity increased up to 4 to 6 days from germination and remained constant thereafter till *ca* 15 days. Attempts by Smith [6] to purify this enzyme from maize leaves were not successful and only a concentration of 2–5-fold could be achieved by removal of inert proteins with alumina C_γ or $Ca_3(PO_4)_2$ gel. The enzyme from groundnut cotyledons was not adsorbed on $Ca_3(PO_4)_2$ gel but it was adsorbed on alumina C_γ gel and could be eluted easily.

pH Optimum

The pH optimum showed a broad range between 5.5 and 8.5 using acetate, Pi and glycine–NaOH buffers. Smith has reported [6] a pH optimum of 6.5–7.5 for the maize leaf enzyme.

Kinetics

The K_m for agmatine was (7.6 ± 0.8) × 10^{-4} M. The enzyme showed optimum activity at 40° and the energy of activation was 22 kcal/mol. The energy of activation for the maize leaf enzyme is 19 kcal/mol [6]. The enzyme retained *ca* 44% activity when exposed to 55° for 10 min and was completely inactivated at 60° under the same conditions.

Table 1. Purification of agmatine iminohydrolase from groundnut cotyledons

Fraction	Total volume (ml)	Total activity (nkat)	Total protein (mg)	Specific activity (nkat/mg protein)	Purification (fold)	Recovery (%)
1. Crude extract	100	15	2600	0.006	1	100
2. Supernatant	82	12	1475	0.008	1	80
3. pH 5.0 supernatant	93	13	205	0.060	11	87
4. $Ca_3(PO_4)_2$ gel supernatant	93	12	150	0.080	14	80
5. Alumina C_γ gel eluate	93	9	45	0.200	33	60
6. DEAE-cellulose eluate:	56	9	4	2.250	375	60

Effect of metal ions

Among the several metal ions tested, only Hg^{2+}, Cu^{2+}, Zn^{2+} and Fe^{3+} inhibited the enzyme activity significantly. Hg^{2+} inhibited the enzyme activity completely at a concentration of 0.2 μM. Cu^{2+} produced complete inhibition at 2.5 μM concentration whereas with Fe^{3+} and Zn^{2+} complete inhibition was obtained at 2 mM.

Inhibition

The enzyme activity was completely inhibited at very low concentrations (25 nM) of *p*CMB which suggests the involvement of -SH groups. Of the several monoamines tested, only tryptamine inhibited the enzyme activity. A 50% inhibition was obtained with 2.5 mM tryptamine. Putrescine, cadaverine, spermidine and spermine also inhibited the enzyme activity. The inhibition was more pronounced with cadaverine and spermidine than with putrescine. Spermine inhibited the enzyme activity only at high concentrations (50% inhibition at 10 mM). The nature of inhibition of cadaverine and spermidine was found to be competitive as revealed by Lineweaver–Burk and Dixon plots. The K_i values for cadaverine and spermidine were 4.1×10^{-3} and 7.5×10^{-4} M, respectively.

EXPERIMENTAL

Plant material. Groundnut seeds (*Arachis hypogea* L. cv Punjab-1) were surface-sterilized with lysol, washed thoroughly with H_2O and then soaked in H_2O for 16 hr. The seeds were germinated at 22° in light in Petri dishes on moist filter papers. *Preparation of* Me_2CO *powder.* Cotyledons from 4 to 6-day-old germinated seeds were ground for 1–3 min with Me_2CO (−20°) in a chilled Waring blender and quickly filtered by suction. The residue was again ground with more Me_2CO and the process was repeated ×5. The powder so obtained was air-dried and stored at 4° in tightly stoppered bottles.

Agmatine iminohydrolase activity was determined by estimating *N*-carbamylputrescine by the method of ref. [7]. The assay system consisted of 0.1 mmol Pi buffer (pH 7.5), 0.6 μmol agmatine and enzyme (0.4 ml) in a total vol. of 4 ml. After incubation at 37° for 1 hr the reaction was terminated by adding 0.5 ml 10% TCA.

Purification. Unless stated otherwise, all purification steps were carried out at 4°. Cotyledon Me_2CO powder (10 g) was homogenized in a chilled pestle and mortar kept over crushed ice using 10 mM Pi buffer, pH 7 and made up to 100 ml. It was then centrifuged at 10000 *g* for 30 min. The supernatant was adjusted to pH 5 with 0.1 M HOAc, allowed to stand for 30 min and centrifuged at 10000 *g* for 20 min. The supernatant was collected and pH adjusted to 7 with 0.1 M NaOH. It was then mixed with $Ca_3(PO_4)_2$ gel (20 mg dry wt/ml) in a gel–enzyme vol. ratio of 2:5, stirred for 30 min and centrifuged at 6000 *g* for 20 min. The supernatant was mixed with alumina C_γ gel (15 mg dry wt/ml) in a gel–enzyme vol. ratio of 3:2. The mixture was stirred for 30 min and centrifuged at 6000 *g* for 20 min. The adsorbed enzyme from the gel residue was eluted with 50 mM Pi buffer, pH 7. The fraction containing the enzyme was applied to DEAE-cellulose column (45 × 1 cm) equilibrated with 50 mM Pi buffer, pH 7. The column was washed with 0.1 Pi buffer, pH 7 and eluted with 0.2 M Pi buffer, pH 7. The enzyme was stored at −5°. The purified enzyme was completely free from amine oxidase.

Protein content of the enzyme fractions was determined by the method of ref. [8].

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