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3-AMINOPROPIONALDEHYDE DEHYDROGENASE OF MILLET SHOOTS

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Key Word Index—*Setaria italica*; Gramineae; millet shoot; 3-aminopropionaldehyde dehydrogenase; 4-aminobutylaldehyde.

Abstract—NAD-dependent 3-aminopropionaldehyde dehydrogenase was partially purified *ca* 18-fold from millet shoots. The purified enzyme (M_r *ca* 70 000) catalysed the oxidation of 3-aminopropionaldehyde (3-APA) to β-alanine. The K_m value was 2.7×10^{-5} M for 3-APA, 4.2×10^{-4} M for 4-aminobutylaldehyde (4-ABA), and 3.8×10^{-5} M for NAD (3-APA case). 3-APA and 4-ABA activity was also found in the shoots of seedlings of prosomillet, maize, oat, barley and pea.

INTRODUCTION

Plant polyamine oxidases (PAOs, EC 1.5.3.3), which convert spermidine (SPD) and spermine (SPM) to 1-pyrroline and 1-(3-aminopropyl)-pyrroline, respectively, with the additional formation in each case of 1,3-diaminopropane (1,3-DAP), have been detected in *Hordeum vulgare* [1, 2], *Zea mays* [1, 3], *Avena sativa* [4, 5], *Setaria italica* [6] and *Eichhornia stricta* [7]. 1,3-DAP fed to *Z. mays* shoots causes an accumulation of β-alanine and SPM, [3-aminopropyl-3-^{3}H(C)] is metabolized to β-alanine [8]. SPD [terminal methylene-^{3}H] in *Lycopersicon esculentum* fruit was catabolized into putrescine and β-alanine [9].

In our previous report we showed that 1,3-DAP is a suicide substrate for diamine oxidase in *Pisum sativum* (DAO, EC 1.4.3.6) [10]. However, DAO from *Setaria italica* is able to oxidize 1,3-DAP to 3-aminopropionaldehyde (3-APA) [11]. This paper describes the partial purification and properties of *Setaria italica* 3-aminopropionaldehyde dehydrogenase (3-APAD) that catalyses 3-APA to β-alanine.

RESULTS AND DISCUSSION

3-APAD from millet shoots was partially purified *ca* 18-fold using $(NH_4)_2SO_4$-fractionation, and DEAE-cellulose and Sephacryl S-100 column chromatography. The results are summarized in Table 1. The optimum pH of the enzyme for both 3-APA and 4-aminobutylaldehyde (4-ABA) was 9.3. The enzyme was most stable between 5.4 and 6.0. The apparent M_r of millet enzyme estimated by Sephacryl S-100 gel filtration was *ca* 70 000.

The substrate specificity is shown in Table 2. 3-APAD had some activity for indole-3-acetaldehyde, but DAO from millet shoots had no activity for tryptamine (data not shown). The stoichiometry of 3-APAD was obtained by measuring the disappearance of 3-APA, and the appearances of β-alanine and NADH. Approximately 1 mol each of β-alanine and NADH were produced from 1 mol of 3-APA.

Millet 3-APAD was inhibited by *p*-chloromercuribenzoate and *N*-ethylmaleimide (Table 3). The results suggested that the enzyme is a thiol enzyme. 4-ABA dehydrogenase (4-ABAD) in *Pisum sativum* [12], and *Pseudomonas* spp. [13, 14] and γ-guanidinobutyraldehyde dehydrogenase (GBAD) having 4-ABAD activity in *Vicia faba* [15] and *Psudomonas putida* [16] have been found. The peaks of 3-APAD activity eluted from the DEAE-cellulose and Sephacryl S-100 columns were coincident with those of 4-ABAD activity. 4-ABAD in *Pseudomonas* spp. had the activity of 3-APAD [14]. It could be that the activity of 4-ABAD in *Pisum* [12], GBAD in *Vicia* and millet 3-ABAD is that of the same enzyme, which accepts either 3-APA or 4-ABA as a substrate. We surveyed the distribution of activities of 3-APAD and 4-ABAD and found them in the shoots of five species of cereals and pea epicotyls (Table 4). The enzyme could function in catabolism of the products of both putrescine (Put) and polyamine oxidation by DAO and PAO. The K_m value was 2.7×10^{-5} M for 3-APA, 4.2×10^{-4} M for 4-ABA and 3.8×10^{-5} M for NAD (3-APA case). Our previous work showed that millet DAO has K_m value each of 9.1×10^{-5} M for 1,3-DAP and 6.3×10^{-4} M for PUT.

β-Alanine, 1.8 μmol and 3-ADA, 0.15 μmol were detected in a 3 ml reaction mixture that had been incubated with 1,3-DAP, 10 μmol, NAD, 2 μmol, millet DAO (2 nkat) and millet 3-APAD (5 nkat) at 37° for 1 hr.

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Table 1. Purification of millet 3-APAD

Step	Volume (ml)	Total protein (mg)	Total activity (nkat)	Specific activity (pkat per mg protein)	Recovery (%)
Crude extract	485	213	140	0.66	100
45–65% $(NH_4)_2SO_4$ precipitate	35	93	118	1.27	84
DEAE-cellulose chromatography	31	37	80	2.16	57
Sephacryl S-100 chromatography	3.7	1.8	22	12.2	16

Table 2. Substrate specificity of millet 3-APAD

Substrates	Relative reaction rate
3-APA	100 (15)* (62)† (1.5)‡
4-ABA	56 (1)*
Glutaraldehyde	32
Indole-3-acetoaldehyde	16
Aminoacetoaldehyde	7
Propionaldehyde	3
Acetoaldehyde	2
Succinic semialdehyde	2

The assay mixture contained 670 μM NAD and 800 μM each aldehyde. *NADP (670 μM), †NAD (67 μM), ‡NADP (67 μM) was used instead of 670 μM NAD in the mixture.

Table 3. Effects of reagents on millet 3-APAD activity

Reagents	Final concn (mM)	Activity (%)
None		100
o-Phenanthroline	0.1	88
	1.0	86
Diethyldithiocarbamate	0.1	95
	1.0	91
Zincon	0.1	87
p-Chloromercuribenzoate	0.1	7
N-Ethylmaleimide	0.1	56
	0.5	28
Phenylhydrazine	0.1	113
	1.0	109
KCN	0.1	93
	1.0	39
1,3-DAP	0.1	108
Put	0.1	114
SPD	0.1	109

The reaction mixture without aminopropionaldehyde was preincubated with reagent at 37° for 5 min. Details are given in the text.

Table 4. 3-APAD activity of the shoots of various cereals

	Activity (pkat mg^{-1} protein)	
Plants	3-APD	4-ABA
Millet	2.6	1.9
Prosomillet	4.5	4.2
Maize	2.4	2.2
Oat	3.6	3.4
Barley	2.7	1.5
Pea	10.8	1.8

Millet shoots contained 32 nmol of 1,3-DAP and 219 nmol of PUT g^{-1} fr. wt. 1,3-DAP was not detected in pea epicotyls which contained PUT (2970 nmol g^{-1} fr. wt (determination by HPLC, see the Experimental Section).

EXPERIMENTAL

Plant. Millet (*Setaria italica* Beauv., cv mochiawa), prosomillet (*Panicum miliaceum* L., cv mochikibi), maize (*Zea mays* L., cv Golden Crossbandam T51), oat (*Avena sativa* L.), barley (*Hordeum vulgare* L., cv Minorimugi) and pea (*Pisum sativum* cv Alaska) were germinated and grown for 5 days in moist vermiculite in plastic trays at 30° (25° for pea) in total darkness. The excised shoots and pea epicotyls were sterilized with 0.1% benzalkonium chloride, and thoroughly washed with tap water.

Plant extracts. The shoots or pea epicotyls (*ca* 5 g) were macerated with 10 ml 0.1 M K-P_i buffer (pH 6.5) containing 15 mM 2-mercaptoethanol and 10% glycerol in a chilled mortar. After centrifugation (25 000 *g*, 15 min), the supernatant was applied on to a PD-10 column and the enzyme was eluted with the same buffer (Table 4).

Chemicals. The following were used: 3-APA diethylacetal (Tokyo Kasei); PD-10 column and Sephacryl S-100 HR (Pharmacia); Zincon (Sigma); DEAE-cellulose,

4-ABA diethylacetal and other chemicals as pure grade (Wako). 3-APA and 4-ABA were prepared from 3-APA- and 3-ABA-diethylacetal, respectively, that had been heated to 100° with 0.1 M HCl in a plugged test tube for 10 min.

Activity of 3-APAD. This was routinely assayed by measuring the increase in A at 340 nm of a reaction mixture. The standard assay mixture consisted of 0.5 ml of 0.5 M glycine–NaOH buffer (pH 9.3), 24 μl of 0.1 M 3-APA or 4-ABA, 0.1 ml of 20 mM NAD and enzyme soln in a total vol. of 3 ml. The assay was initiated by the addition of substrate and A_{340} measured during incubation at 37° for 10 min. Enzyme activity is presented as the amounts of formed NADH (mol sec^{-1}) in the standard assay system.

Millet 3-APAD. All the operations were carried out at 4°. The washed shoots (100 g) were ground in a Waring blender with 200 ml 0.1 M K-P_i buffer (pH 6.5) containing 15 mM 2-mercaptoethanol and 10% glycerol and the resulting slurry was filtered through a layer of cotton cloth, then centrifuged at 10 000g for 15 min. The supernatant was fractionated stepwise with solid $(NH_4)_2SO_4$, and the fraction obtained between 45% and 65% satn was collected by centrifugation. The ppt. was dissolved in 30 ml of 0.1 M K-P_i buffer (pH 6) containing 15 mM 2-mercaptoethanol and 10% glycerol (buffer A) and centrifuged at 10 000g for 10 min. The supernatant was freed of $(NH_4)_2SO_4$ by passing the soln through a Sephadex G-25 column (2.2 × 50 cm) equilibrated with buffer A. The active fraction (35 ml) was applied to a DEAE cellulose column (1.5 × 24 cm) equilibrated with buffer A. The enzyme was eluted with 100 ml of a linear gradient of buffer A to buffer A containing 0.7 NaCl. The active fractions were collected and made 70% satn with solid $(NH_4)_2SO_4$. The ppt after centrifugation at 10 000g for 10 min was added 0.5 ml of buffer A and centrifuged at 10 000g for 10 min. The supernatant was applied to a Sephacryl S-100 HR column (1.5 × 117 cm) equilibrated with buffer A. The active fractions were collected and used as the purified enzyme.

Millet DAO. DAO from millet shoots was prepared by the method of ref. [11].

Stoichiometry. After incubation of the standard assay mixture used 0.5 M borate–NaOH (pH 9.3) instead of 0.5 M glycine–NaOH, 0.1 vol. (v/v) 50% TCA was added to the mixture and centrifuged. The supernatant was applied to the amino acid analytical system (Hitachi Amino Acid Analyzer 853, elution buffer PS-SET-1). 3-APA and β-alanine each were eluted in the same positions as histidine and galactosamine, respectively.

In the case of incubation with 1,3-DAP, the mixture consisted of 0.5 ml of 0.2 M Tris–HCl (pH 8.0), 0.1 ml of 0.1 M 1,3-DAP, 0.1 ml of 20 mM NAD and appropriate amounts of millet DAO and APAD solns in a total vol. of 3 ml. β-Alanine and 3-APA in the incubated mixture were determined according to the procedures described above.

Determination of 1,3-DAP and Put in millet shoots and pea epicotyls. Plant material was macerated with 10 vol. (w/v) 5% $HClO_4$ and centrifuged. The supernatant was dansylated by the method of ref. [17]. Sepn of the dansylated 1,3-DAP and Put was performed by HPLC, a Shimadzu LC system equipped with LC-10A pump, RF-535 fluorescence HPLC monitor (Ex. 365 nm, Em. 510 nm), a column; Shim-pack CLC-ODS (M) 4.6 × 250 mm, solvent system; MeOH–CH_3CN–H_2O (14 : 1 : 5), and flow rate; 0.8 ml min^{-1}.

Protein. Determined according to ref. [18] with bovine serum albumin as the standard.

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