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SHORT REPORTS

POLYAMINE OXIDASE OF MILLET SHOOTS

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Key Word Index—*Setaria italica*; Gramineae; millet shoot; polyamine oxidase; flavoprotein.

Abstract—Polyamine oxidase was purified *ca* 168-fold from the acetone powder extract of millet shoots. The light yellow enzyme had maximum absorption at 278, 380 and 460 nm. The absorption at 380 and 460 nm was decreased by the addition of spermidine. The enzyme (M_r *ca* 80000) showed a high specificity for spermine and spermidine (K_ms 6 $\times 10^{-5}$ M and 5×10^{-7} M respectively). The enzyme was inhibited by quinacrine and acriflavine.

INTRODUCTION

Although polyamine oxidases (PAOs) have been detected in several species of the Gramineae [1], the enzyme has been characterized only in barley, oat and maize [2, 3]. This paper describes the partial purification of PAO from millet shoots and compares its properties with those of other plant PAOs.

RESULTS AND DISCUSSION

PAO from millet shoots was purified 168-fold from the acetone powder extract according to the method of ref. [4]. The results are summarized in Table 1. The optimal pH of the enzyme for both spermine and spermidine was 6.5, like the oat leaf PAO [2]. The apparent M_r of the millet enzyme estimated by Sephadex G-100 gel filtration was 80000. Spermine and spermidine were substrates, but cadaverine, putrescine, tryptamine and tyramine were not oxidized. K_m values were 6×10^{-5} M for spermidine and 5×10^{-7} M for spermine, calculated from Lineweaver–Burk plots. Enzyme activity (under pH 6.5) for both substrates was inhibited 97 and 100% by quinacrine and acriflavine (each at 1 mM), respectively as found in maize PAO [4]. On the other hand, semicarbazide, *o*-phenanthroline and *p*-hydroxymercuriphenylsulphonate (pH 6.5; each at 1 mM) were ineffective. The absorption spectrum of the enzyme preparation (final step in Table 1) is characterized by A_{max} at 460, 380 and 278 nm. These results agree with the previous finding for the maize PAO [4]. The A at 460 and 380 nm decreased upon the addition of spermidine, but not with putrescine, suggesting that the reduction of flavine is involved in the catalytic activity.

EXPERIMENTAL

Plant materials. The millet seeds (*Setaria italica* cv. mochiawa) were germinated in moist vermiculite at 25° for 6 days in the dark. The excised shoots were sterilized with 1% benzalkonium chloride, and thoroughly washed with deionized H_2O.

Partial purification. The washed shoots (500 g) were ground in a Waring blender with 1.5 l. of cold Me_2CO (−10°) by the method of ref. [5]. The Me_2CO powder (*ca* 20 g) was blended with 0.5 M KH_2PO_4 (1 l.) and the resulting slurry was filtered through a layer of cotton cloth, then centrifuged at 10000 *g* for 15 min. The supernatant diluted two-fold with deionized H_2O, was stirred with CM-Sephadex equilibrated with 0.25 M KH_2PO_4 for 1 hr (supernatant/CM-Sephadex = 1 l./100 g wet wt), then the CM-Sephadex was recovered on a Büchner funnel. After thorough washing with 0.25 M KH_2PO_4, the CM-Sephadex was packed into a glass cylinder, and the enzyme was eluted with 1 M KH_2PO_4. The active fractions obtained from CM-Sephadex were concd to 2.6 ml in a collodion bag by the suction pump. The concd enzyme was applied to a Sephadex G-100 column (1.5 × 70 cm) equilibrated with 0.5 M KH_2PO_4 and subsequently eluted with 0.5 M KH_2PO_4. The active fractions were concd to 2.6 ml by the procedure given above.

The PAO activity was determined using a Clark oxygen electrode at 30°. The standard assay mixture contained (in a total vol. at 1.6 ml) 0.6 ml of 0.5 M KPi buffer pH 6.5, 250 μg of beef liver crystalline catalase, 0.3 ml of spermidine or spermine (each 50 mM) and enzyme soln. The total vol. was adjusted with deionized H_2O. The reaction was initiated by the addition of

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Table 1. Purification of millet polyamine oxidase

Step	Volume (ml)	Total protein (mg)	Total activity (nkat)	Specific activity (nkat/mg protein)	Purification (fold)	Yield (%)
Acetone powder extract		2250	2480	1.1	1	100
Centrifugation	750	468	1920	4.1	3.7	78
CM-Sephadex	2.6	20	562	28.1	25.5	23
Sephadex G-100	2.6	6.2	1147	185	168	46

polyamines and the enzyme activity was calculated from the initial rate of O_2-consumption. As an alternative assay used for the determination of pH curves of spermidine oxidation, pyrroline, the oxidation product of spermidine, was determined according to the method of ref. [6]. The standard incubation mixture consisted of 1 ml of 0.5 M KPi buffer pH 6.5, 0.5 ml of 50 mM spermidine, 0.2 ml of 0.1% *o*-aminobenzaldehyde (in EtOH) and enzyme soln. Total vol. was adjusted to 1.5 ml with deionized H_2O. The reaction was initiated by the addition of spermidine, and the reaction (5 min) was stopped by the addition of 1 ml of 50% TCA and 4 ml of deionized H_2O. After filtration, *A* was estimated at 435 nm.

The M_r *of the enzyme* was determined with a molecular sieve according to ref. [7]. Trypsin inhibitor (chicken egg white; 28000), BSA (136000), cytochrome *c* (12400) and γ-globulin (165000) were used to calibrate the Sephadex G-100 column.

Protein was determined according to ref. [8].

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