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POLYAMINE OXIDASE FROM *ZEA MAYS* SHOOTS

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Key Word Index—*Zea mays*; Gramineae; maize; spermidine; spermine; polyamine oxidase; acridine compounds; FAD.

Abstract—Polyamine oxidase of maize shoots purified 10-fold had a pH optimum of 6·3 with spermidine as substrate, and K_m of 6×10^{-4} M. The enzyme was inhibited by the acridine compounds quinacrine, 6,9-diamino-2-ethoxyacridine and acriflavin, but carbonyl reagents, typical thiol inhibitors and copper-binding agents were without effect. Inhibition by quinacrine was reversed by FMN and FAD. Furthermore, about 50% of the activity of the apoenzyme was restored by the addition of FAD, but not by FMN or riboflavin, indicating that the maize polyamine oxidase is an FAD-dependent flavoprotein.

INTRODUCTION

THE PROPERTIES of a polyamine-specific oxidase found in the shoots of various Gramineae,[1,2] differ strikingly from those of the diamine oxidase (E.C. 1.4.3.6) of various Papilionaceae.[3] In a previous paper,[4] we reported that maize polyamine oxidase is inhibited by the acridine compounds atebrin, 6,9-diamino-2-ethoxyacridine and acriflavin and that the inhibition by atebrin is reversed by FMN and FAD. We have now established that this enzyme requires FAD. The present paper also describes some other properties of maize polyamine oxidase.

RESULTS

Partial Purification of Maize Polyamine Oxidase

A 10-fold purification was achieved using the procedure given in Experimental (Table 1). Activity was inhibited by treatment with ammonium sulphate, as also reported by Smith,[2] and this inhibition was irreversible. Potassium dihydrogen phosphate is an efficient solubiliz-

TABLE 1. PURIFICATION OF MAIZE POLYAMINE OXIDASE

Fraction	Total vol. (ml)	Units/ml	Total units	Nitrogen (mg/ml)	Spec. act. (unit/mg)	Recovery (%)
1 Crude extract	1530	3·8	5740	0·89	4·2	100
2 Particles	—	6·3	—	1·49	4·2	—
3 KH_2PO_4 (0·5 M) extract	110	11·3	1240	0·43	26·0	21·6
4 Sephadex G25 treated	50	23·1	1160	0·52	44·6	20·1

A unit of activity is defined as the amount of enzyme which causes 10 μl O_2 uptake in 10 min under standard conditions.

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ing agent for the particulate enzyme, and the resulting solution has a pH close to 5, a value at which this enzyme shows optimal stability.

Substrate Specificity

Spermine was oxidized much less readily than spermidine by the partially purified enzyme and by the particle suspension on measuring the O_2 uptake at pH 6·3. Cadaverine, putrescine, histamine, tryptamine, *n*-butylamine and L-lysine were not oxidized by either preparation.

pH Optimum

The effect of pH on the rate of oxidation of polyamines by the enzyme was estimated on the basis of the O_2 uptake and the *o*-aminobenzaldehyde reaction (see Experimental). The enzyme was found to have maximal activity at pH 6·3 with spermidine, and pH 5·5 with spermine (Fig. 1).

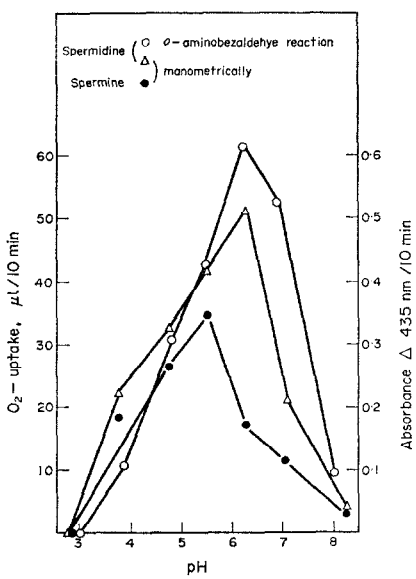

FIG. 1. EFFECT OF pH ON THE POLYAMINE OXIDASE ACTIVITY.

Standard assay conditions were used, except that Tris-HCl buffer was employed for pH 8·0 and citrate–phosphate buffer for pH 3–5.

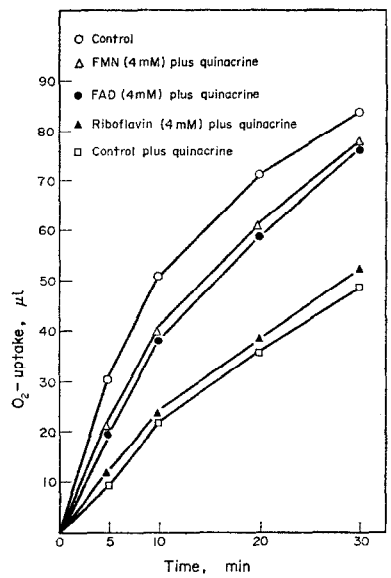

FIG. 2. REACTIVATING EFFECT OF FAD AND FMN AFTER INHIBITION BY QUINACRINE.

Standard manometric assay conditions were used (see Experimental).

Michaelis Constant

The effect of the spermidine concentration on the reaction velocity was determined with the *o*-aminobenzaldehyde reaction. Enzyme activity was linear with time up to 10 min, and was proportional to protein concentration. The K_m value for spermidine calculated from a Lineweaver–Burk plot was 6×10^{-4} M.

Effect of pH and Temperature on Enzyme Stability

The enzyme was most stable near pH 5 (citrate–phosphate buffer); losing 18% of its activity in 48 hr at 30°. At pH 3·5, 6·3 and 8·5, activity loss in 48 hr was 22%, 40% and 85%

TABLE 2. EFFECT OF INHIBITORS ON THE OXIDATION OF SPERMIDINE BY MAIZE AND PEA ENZYMES

Inhibitor	Conc. (mM)	Inhibition in 10 min (%) Maize enzyme pH = 6·0	Pea enzyme pH = 7·0
(a) *Carbonyl reagents*			
Hydroxylamine	2	0	99
Aminoguanidine	1	0	99
Semicarbazide	2	2	100
Parnate (*trans*-2-phenylcyclopropylamine)	0·2	0	4
(b) *Chelating reagents*			
Xanthogenate	1	0	99
Diethyldithiocarbamate	1	0	98
o-Phenanthroline	2	0	99
Cuprizone	0·2	0	50
Thiocyanate	2	28	14
Thiocyanate	2	0 (pH = 5·0)	—
Azide	2	0	32
Azide	2	4 (pH = 5·0)	—
(c) *Thiol reagents*			
p-Hydroxymercuriphenylsulfonate	1	0*	0†
N-Ethylmaleimide	1	0*	0†
Phenylmercuricacetate	0·2	0*	70†
(d) *Acridine compounds*			
Quinacrine	2	56	17
Rivanol (6,9-Diamino-2-ethoxyacridine)	2	89	29
Acriflavin (neutral)	2·5 (mg)	91	31

* Preincubation 10 hr at 30°.
† Preincubation 20 hr at 30°.
Standard manometric assay conditions were used. Protein content of pea enzyme was 6·7 mg/ml.

respectively. The enzyme was relatively heat-stable; 30 min at 50° and 60° resulted in 20% and 40% loss of activity respectively: above 70°, however, the enzyme was rapidly inactivated with 95% activity lost in 5 min.

Effect of Inhibitors and Cofactors

The effect of various inhibitors on the oxidation of spermidine by maize and pea enzyme was tested by manometry, and the results are given in Table 2 and Fig. 2. In addition, the

TABLE 3. FAD REQUIREMENT OF MAIZE POLYAMINE OXIDASE

Enzyme preparation	Flavin	Final conc, (mM)	Activity (uptake of O_2 in μl) 10 min	30 min
Native enzyme	—	—	86	149
APO-enzyme	—	—	22(26*)	39(26*)
APO-enzyme	FAD	0·2	41(48*) (86†)	80(54*) (105†)
APO-enzyme	FMN	0·2	22	40
APO-enzyme	Riboflavin	0·2	22	38
APO-enzyme (boiled)	FAD	0·2	0	0

* % of original activity.
† % increase over control.
Standard manometric assay conditions were used, except that catalase was omitted.

apoenzyme of the maize polyamine oxidase was resolved by the method of Tabor and Kellogg[5] (see Experimental) and was found to be partially reactivated by 0·2 mM FAD (Table 3). Addition of FMN or riboflavin was without effect.

DISCUSSION

The experiments described in this paper show that maize shoots contain an enzyme which oxidizes the polyamines, spermidine and spermine, but which has no action on a variety of other amines. Unlike other amine oxidases in higher plants,[6–12] the maize enzyme was not appreciably inhibited by copper–chelators or carbonyl reagents. Parnate (*trans*-2-phenylcyclopropylamine), which is a potent inhibitor of animal monoamine oxidase[13] does not inhibit the maize polyamine oxidase or the pea seedling diamine oxidase. Maize polyamine oxidase was inhibited somewhat by thiocyanate but this inhibition was lost at pH 5, suggesting that there is no interaction with a heavy metal such as molybdenum[14] or iron.[15] Phenylmercuricacetate inhibited the pea enzyme but probably not in its capacity as a thiol reagent, the other thiol reagents tested were ineffective with both enzymes. The maize enzyme was markedly inhibited by acridine compounds such as quinacrine, 6,9-diamino-2-ethoxyacridine and acriflavin. Quinacrine is usually considered to be a specific inhibitor of flavin containing enzymes.[5,16–20] As shown in Fig. 2, the inhibition caused by preincubation with 2 mM quinacrine for 30 min was reversed by adding FMN or FAD. In order to investigate the nature of this inhibition, the enzyme was treated by a method known to resolve flavin prosthetic groups from the holoenzyme of amine oxidase.[5] The resulting inactive preparation of the maize polyamine oxidase was reactivated by the addition of FAD (Table 3), suggesting that FAD is essential for enzyme activity. In this respect, maize enzyme seems to be similar to the spermidine dehydrogenase of the bacterium *Serratia marcescens*.[5,21] However, maize enzyme differs from *Serratia* enzyme in that it does not reduce any added electron carriers such as 2,6-dichlorophenolindophenol, nitroblue tetrazolium plus phenzine methosulfate, thionine, toluylene blue and methylene blue using a conventional Thunberg technique (unpublished).

EXPERIMENTAL

Plant material. Maize seedlings (*Zea mays* L. cv. Goldencross Bantam T51) and pea seedlings (*Pisum sativum* L. cv. Alaska) were grown in moistened vermiculite at 25° in the dark for 7–8 days.

Enzyme preparation. All operations were performed at 4°. The tops (900 *g*) which were surface sterilized for 5 min in 0·1% benzalkonium chloride soln were ground in a large mortar with 900 ml of deionized H_2O

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containing 1 mM EDTA and 10 mM 2-mercaptoethanol. The homogenate was filtered through cotton cloth and the filtrate was centrifuged at 5000 *g* for 30 min. The ppt. (particle fraction) was ground in a mortar with 110 ml of 0·5 M KH_2PO_4 soln. The slurry was stirred in a beaker overnight, and centrifuged at 10000 *g* for 30 min. Sephadex G25 (coarse; 20 g) was added to the supernatant (110 ml), and the mixture was agitated for 10 min. The clear soln obtained after filtering through a filter paper on a Buchner funnel was used as 'maize enzyme'. Partially purified amine oxidase from pea cotyledons was prepared according to Mann.[22]

Preparation of apoenzyme.[5] Bovine serum albumin (8 mg) was added to 4 ml of maize enzyme soln (containing 4 mg protein per ml) at 4°, and to this was added 4 ml of saturated KBr soln, and 8 ml of saturated $(NH_4)_2SO_4$ soln, adjusted to pH 2·2 with 1 N H_2SO_4. The resulting ppt. was centrifuged for 10 min at 10000 *g* and immediately dissolved in 4 ml of 0·2 M phosphate buffer (pH 7). The supernatant was used as the apoenzyme.

Determination of enzyme activity. The O_2 uptake was estimated by a Warburg manometer at 30°. The total vol. of the reaction mixture was 2·5 ml, and 0·2 ml of 20% KOH was placed in the center cup. No O_2 uptake by the maize and pea enzyme preparations could be detected in the absence of substrate. The standard assay system was as follows: 0·1 ml (0·4 mg protein) of enzyme soln, 1 ml of 0·1 M phosphate buffer (pH 7), 0·1 ml of beef liver crystalline catalase (250 μg/0·1 ml) and 0·5 ml of spermidine (3 HCl) soln (50 mM). For the colorimetric estimation of enzyme activity, used for the determination of the K_m and pH curves, Δ′-pyrroline, the product of spermidine oxidation, was assayed by a method similar to that described by Holmstedt *et al.*[23] The reaction mixture consisted of 0·5 ml of 50 mM spermidine (3 HCl) soln, 1 ml of 0·1 M phosphate buffer (pH 7), 0·1 ml of enzyme soln, 0·2 ml of 0·1% *o*-aminobenzaldehyde (in EtOH) and 0·1 ml of catalase suspension (250 μg/0·1 ml). Total vol. was adjusted to 2·5 ml with deionized H_2O. The mixture was incubated at 30° with shaking, and the reaction 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. Data for both methods were calculated from the readings recorded in the first 10 min after the start of the reaction. Protein was determined by the method of Lowry *et al.*,[24] with bovine serum albumin as the standard. Nitrogen determinations were carried out according to the method of Katsumata.[25]

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