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PROPERTIES OF THE POLYAMINE OXIDASE FROM THE CELL WALL OF MAIZE SEEDLINGS

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Key Word Index—*Zea mays*; Gramineae; maize; cell walls; polyamine oxidase.

Abstract—Polyamine oxidase was purified 4.8-fold from the cell wall extract of maize seedlings using only two chromatographic steps. The enzyme (M_r *ca* 53 000) which had a specific activity of 700 nkat/mg at 37° showed a similar pH optimum (6.5) with both spermidine and spermine as substrates. For spermine and spermidine the K_ms were 18 and 22 μM respectively. The light yellow enzyme had absorption maxima at 278, 380 and 456 nm. The addition in anaerobic conditions of equimolar amounts of substrates induced a decrease of *A* at 380 and 456 nm, while reoxygenation of the enzyme restored the native spectrum. The enzyme contained 2.5% sugar, mainly as arabinose.

INTRODUCTION

Although the polyamines spermine (Sn) and spermidine (Sd) are widely distributed in higher plants [1], the polyamine oxidases (PAOs) have been detected mainly in the Gramineae [2] and recently in water hyacinth [3]. Plant PAO has been purified to homogeneity from maize [4] and water hyacinth [3] and partially purified from barley, oat and millet [2, 5, 6]. In the present paper we describe a rapid and efficient purification procedure of PAO from maize cell walls using only two chromatographic steps.

RESULTS AND DISCUSSION

Purification

Most of the activity (90%) was associated with cell walls from which the enzyme could be solubilized by washing in 0.5 M KH_2PO_4. As the specific activity of cell wall extract was very high, this fraction was utilized for purification of the enzyme on CM-cellulose and hydroxylapatite columns (see Experimental). By this procedure a five-fold purification of the enzyme was achieved with a recovery of *ca* 80% (Table 1). The homogeneity of the purified enzyme was evaluated by analytical gel electrophoresis in native (pH 4.3) and denaturing (SDS) conditions: the results showed a single band after staining with Coomassie Blue.

Maize PAO obtained by this procedure showed properties similar, but not identical to those observed previously [4]. The enzyme which had a specific activity of 700 nkat/mg at 37°, showed a M_r of *ca* 53 000 by gel filtration (Bio Gel P200 or HPLC) or by SDS electrophoresis. Like the oat (*Avena sativa*) leaf PAO [2] it was equally active with Sd and Sn, and both substrates had the same pH optimum (6.5), and like the barley PAO [7], maize enzyme is characterized by a relatively large K_m for O_2 (*ca* 10^{-4} M). The apparent K_m values calculated from Lineweaver–Burk plots were very similar for Sn (18 μM) and Sd (22 μM), and are in agreement with those previously reported [4].

The sugar content of the enzyme was *ca* 2.5%, mainly represented as arabinose. The absorption spectrum of the native enzyme showed a three banded spectrum, typical for oxidized flavoprotein, with maxima at 278, 380 and 456 nm. The addition of equimolar amounts of substrates in anaerobic conditions induced the oxidation of enzyme as shown by the decrease of the *A* in the visible range at 380 and 456 nm, while reoxygenation of the enzyme restored the spectrum, confirming the involvement of flavin in the catalytic cycle (Fig. 1).

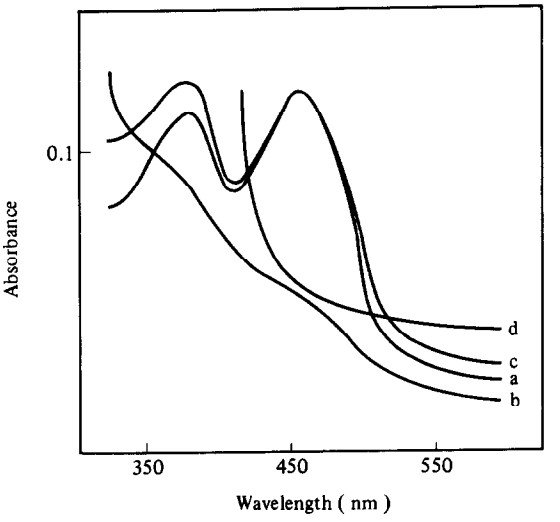

Fig. 1. Absorbance spectra of maize PAO: effect of adding spermidine to oxidized enzyme, and oxygenation of the reduced form. Absorbance spectra were recorded in anaerobiosis with 3 ml of 10 μM PAO in 0.1 M K-Pi pH 6.5 using a Thunberg type cuvette. (a) Native enzyme in anaerobic conditions; (b) native enzyme in anaerobic conditions +20 μM spermidine; (c) 'b' enzyme after reoxygenation; (d) enzyme + sodium dithionite.

Table 1. Purification of PAO from maize shoots using 800 g of 12-day-old dark grown seedlings as starting material

	Total volume (ml)	Total protein (mg)	Total activity (nkat)	Specific activity (nkat/mg protein)	Purification (fold)	Recovery (%)
Cell wall extract	1500	68	10000	147	–	100
Carboxymethyl-cellulose	150	24	8800	365	2.5	88
Hydroxylapatite	8	11	7700	700	4.8	77

Although the present purification procedure resulted in only a five-fold increase in specific activity, the enzyme was electrophoretically homogeneous and displayed a very high specific activity. These results confirm the localization of PAO in the cell wall [8] and indicate the use of an acidic buffer (KH_2PO_4) for the extraction of the enzyme from this site. The good recovery (*ca* 80%) of PAO and the rapidity of the purification method described above, makes it competitive with others already published. Our results suggest that this procedure is highly suitable for large scale preparation of maize PAO.

EXPERIMENTAL

Plants. Commercial seeds of maize (*Zea mays* cv 'Logos') were soaked for 2 hr in aerated tap water and grown in moistened vermiculite for 12 days in the dark at 25°.

Homogenate. Shoots (800 g) of etiolated maize seedlings were homogenized in a Waring blender with 4 vols of cold H_2O, and the homogenate was strained through cheese cloth. The solid residue was then washed $\times 3$ with 3 vols of cold H_2O, and two times with 2 vols of 0.1 M KH_2PO_4. The enzyme was then eluted with 1 vol. of 0.5 M KH_2PO_4. The suspension was pressed through cheese cloth, and centrifuged at 13000 *g* for 30 min at 0°. The pellet was discarded and the supernatant used for the purification procedure.

Purification of PAO. The cell wall extract was diluted 2.5-fold with H_2O and applied to a carboxymethyl cellulose (CM 52) column (80 ml) equilibrated with 0.2 M KH_2PO_4. The column was then washed with the same buffer until the A_{280} was at the background level and then eluted with 0.1 M NaCl in 0.25 M KH_2PO_4. The active fractions were applied directly to a hydroxylapatite column (10 ml) pre-equilibrated with 0.1 M NaCl in 0.25 M KH_2PO_4 and washed with the same equilibration soln. The enzyme was eluted with 0.5 M KH_2PO_4 buffer, pH 5.5.

Assays. PAO activity was estimated at 37° in a Hansatech Oxigraph equipped with a Clark electrode, according to ref. [9]. Protein was estimated by the method of ref. [10] with bovine IgG as standard and the pH optimum was determined in 0.2 M KPi (4–8) buffer. The M_r was determined using molecular sieving method according to ref. [11] on a Bio-Gel P200 column, and SDS electrophoresis carried out according to ref. [12]. The presence of sugar was investigated after hydrolysis of the enzyme in 2 M TFA; the monosaccharides were detected and identified as dansylhydrazine derivatives according to ref. [13]. Absorption spectra were recorded by a PU 8820 (Philips) spectrophotometer. Anaerobic experiments were conducted at 25° in a Thunberg type cuvette where anaerobic additions of various reagents can be made with a syringe through a rubber seal.

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