Phytochemistry, Vol. 28, No. 8, pp. 2045–2046, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00

PURIFICATION AND CHARACTERIZATION OF OAT POLYAMINE OXIDASE

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(*Received* 27 *January* 1989)

Key Word Index—*Avena sativa*; Gramineae; oat; polyamine oxidase.

Abstract—Polyamine oxidase from oat shoots was purified to homogeneity by the criteria of polyacrylamide gel electrophoresis in native and denaturing conditions. The purified yellow enzyme had an A_{max} at 276, 370 and 452 nm. The A_{452} and A_{370} decreased upon addition in anaerobiosis of spermine and spermidine or dithionite, but not putrescine. The enzyme had a M_r of *ca* 63 000 and a specific activity of 1200 nkat/mg at 37° with spermidine as substrate. It showed a K_m for spermine and spermidine of 6.0 μM and 9.5 μM respectively, the same pH optimum (6.8) for both substrates and was inhibited by N^1-acetylspermine.

INTRODUCTION

The polyamine oxidases (PAOs) have been detected mainly in the Gramineae [1] and recently in the water hyacinth [2]. This enzyme, which is particularly active in oat [3] has been well characterized in barley [4], maize [5, 6] and millet [7]. This paper describes a simple method for purifying oat PAO and some properties of the enzyme.

RESULTS AND DISCUSSION

PAO from green shoots of 15-day-old oat seedlings was purified from the acetone powder extract; the enzyme could be solubilized by 0.1 M K-Pi buffer pH 5. A 16-fold purification was achieved in three chromatographic steps (hydroxylapatite, octyl-sepharose, carboxymethylcellulose) and the results are summarized in Table 1. Polyacrylamide gel electrophoresis in native (pH 4.3) and denaturing (SDS) conditions of the PAO obtained by the reported purification procedure shows a single band, demonstrating the homogeneity of the preparation.

Oat PAO shows similar, but not identical properties to those previously observed [3]. The enzyme, which has a specific activity of 1200 nkat/mg at 37°, shows a M_r of *ca* 63 000 by SDS-PAGE, is equally active with spermine and spermidine, and has the same pH otpimum (6.8) for both substrates. The apparent K_m values calculated from Lineweaver–Burk plots are similar for spermine (6 μM) and spermidine (9.5 μM). Enzyme activity is inhibited *ca* 50% by acridine orange at 5 μM, while copper ligands (KCN, diethyldithiocarbamate), carbonyl group-directed reagent (phenylhydrazine) and diamine oxidase inhibitors (aminoguanidine, anserine, carnosine) are ineffective. No activity is found with N^1 acetylspermidine and N^1 acetylspermine, which are extremely good substrates for mammalian polyamine oxidases [8]. Moreover, 2 μM N^1 acetylspermine completely inhibits the enzyme, as already found for barley PAO with the spermidine analogue $[NH_2(CH_2)_3NH(CH_2)_{10}NH_2]$ [4]. The absorption spectrum of the native yellow enzyme is typical for an oxidized flavoprotein and is characterized by A_{max} at 276, 370 and 452 nm.

As previously shown for maize PAO [6], the addition in anaerobiosis of equimolar amounts of spermine and spermidine, but not putrescine, cadaverine and N^1acetylspermine causes a decrease of the A at 370 and 452 nm, while reoxygenation restores the spectrum; this result suggests that the reduction of flavine is involved in the catalytic activity.

EXPERIMENTAL

Chemicals. Anserine (β-alanyl-1-methylhistidine), carnosine (β-alanyl-histidine), N^1 acetylspermine and N^1 acetylspermidine

Table 1. Purification of PAO from oat shoots

Chromatographic step	Total volume (ml)	Total protein (mg)	Protein (mg/ml)	Activity (nkat/ml)	Total activity (nkat)	Specific activity (nkat/mg prot)	Recovery (%)
Acetone powder extract	400	88	0.220	16.5	6600	75	100
Hydroxylapatite. eluate	250	38	0.150	19	4750	127	72
Octyl-Sepharose eluate	220	3.1	0.014	10.8	2380	773	36
Carboxymethyl-cellulose eluate	34	1.8	0.052	61.6	2100	1190	32

were from Sigma; hydroxylapatite and carboxymethylcellulose were from Bio-Rad; octyl-Sepharose was from Pharmacia. All other chemicals were obtained as pure commercial products.

Plant material. Oat seeds (*Avena sativa* L.) were soaked for 12 hr in aerated tap water and then grown in moistened vermiculite for 15 days at 25° (14 hr/day; 300 μmol/sec/m^2).

Purification. Shoots (200 g) were extracted in a blender with 3 vols of cold Me_2CO by the method of ref. [9]. The Me_2CO powder was blended with 0.1 M K-Pi, pH 5 (400 ml) and the resulting slurry strained through cheesecloth and centrifuged at 20000 *g* for 30 min at 4°. The pellet was discarded and the supernatant applied to a hydroxylapatite column (10 ml) pre-equilibrated with 0.1 M K-Pi pH 5. The column was then washed with the same equilibration soln. Elution was carried out with a linear gradient from 0.1 M K-Pi to 0.1 M K-Pi + 2 M NaCl pH 5. The active fraction (0.1 M K-Pi + 1.25 M NaCl) was made 3 M by adding NaCl and applied to an octylsepharose column pre-equilibrated with 0.1 M K-Pi + 3 M NaCl pH. 5. The enzyme was eluted with a linear gradient from 0.1 M K-Pi + 3 M NaCl to 0.1 M K-Pi + 0.5 M NaCl. The active fractions (0.1 M K-Pi + 1.5 M NaCl) were dialysed overnight against 0.05 M K-Pi pH 5 and adsorbed onto a carboxymethylcellulose column (80 ml), pre-equilibrated with 0.05 M K-Pi pH 5. PAO was eluted with 0.2 M K-Pi pH 5.

Assays. PAO activity was estimated at 37° in a Hansatech Oxigraph equipped with a Clark electrode, according to ref. [10]. Protein content was estimated by the method of ref. [11] with bovine IgG as standard and the pH optimum was determined in 0.2 M K-Pi (pH range 4–8) buffer. SDS-PAGE was carried out according to ref. [12]. Native electrophoresis was performed according to refs [2, 13]. Absorption spectra were recorded by a PU 8820 (Philips) spectrophotometer. Anaerobic experiments were performed at 25° in a Thunberg type cuvette where anaerobic additions of various reagents can be made with a syringe through a rubber seal.

Acknowledgements—This research was supported by funds for scientific research from Ministero della Pubblica Istruzione, Italy.

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