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OXIDATION OF ACETYLPOLYAMINES BY MAIZE POLYAMINE OXIDASE

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Key Word Index—*Zea mays*; Poaceae; maize; polyamine oxidation; polyamine oxidase; acetyl polyamine; polyamine interconversion pathway.

Abstract—The oxidation of acetylpolyamines by cell wall polyamine oxidase from maize shoots was investigated. The purified enzyme catalysed the oxidation of N^1-acetylspermine, N^1-acetylspermidine, and N^8-acetylspermidine at the same optimal pH (6.5), but with lower relative velocities and higher K_m than those found for spermine and spermidine oxidation. The enzyme cleaved N^1-acetylspermine and N^8-acetylspermidine, at the same positions as in spermine and spermidine oxidation, with the production of H_2O_2, 1,3-diaminopropane and the corresponding aminoaldehydes. Polyamine oxidase was quickly inactivated by catalysis, and the aminoaldehyde derived from N^1-acetylspermine behaved as a competitive inhibitor of the enzyme ($K_i = 20\ \mu M$). These findings suggest that cell wall polyamine oxidase from maize shoots does not effect the interconversion pathway of acetylpolyamines found in vertebrates.

INTRODUCTION

The FAD containing polyamine oxidases (PAOs; EC 1.5.3.3) catalyse the oxidation of polyamines at the secondary amino groups [1]. Vertebrate PAOs transform spermidine (Spd) and spermine (Spm), and more efficiently their N^1-acetyl derivatives, into putrescine (Put) and Spd, respectively, plus propanal or acetamidopropanal, which has been named the interconversion pathway of polyamine degradation [2]. 1,3-Diaminopropane (DAP), 4-aminobutyral or 3-aminopropyl-4-aminobutyral are the products of Spd and Spm oxidation catalysed by PAOs from plants [3], bacteria [2] and protozoa (*Acanthamoeba castellanii*) [4]. As these compounds cannot be converted to other polyamines, PAO is considered to be involved in terminal catabolism of polyamines in these taxa. Moreover, N^1-acetyl Spm, which is the best substrate for vertebrate PAOs, acts apparently as a non competitive inhibitor for maize and oat enzymes [5]. As the existence of a possible Spd–Put conversion has been recently proposed in tobacco thin layer explants [6], maize roots [7], root cultures of *Senecio vulgaris* [8], callus lines of *Picea abies* [9] and *Helianthus tuberosus* chloroplast [10], and as acetyl derivatives of polyamines have been found in the latter system [10], we have studied the oxidation of acetylpolyamines by PAO purified from maize cell walls, an enzyme which we have characterized [5]. Previous studies suggested that this enzyme may be involved in the production of H_2O_2 in the apoplast thus implying a role in crosslinking and defence [11]. We have chosen maize PAO for this investigation in order to determine whether a role in polyamine interconversion may be also attributed to this enzyme.

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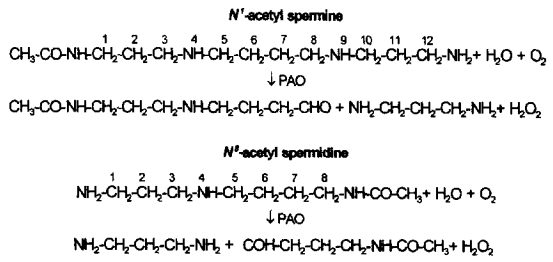
RESULTS AND DISCUSSION

Pure maize PAO obtained from a cell wall preparation (mPAO) catalyses the oxidation of acetylpolyamines as deduced by O_2 depletion under standard conditions for polyamine oxidation [12]. The optimal pH for the degradation of N^1-acetylSpm, N^1-acetylSpd and N^8-acetylSpd was very similar to that found for Spm and Spd oxidation (6.5). The substrate specificities for several kinds of acetyl polyamines were investigated under standard conditions at pH 6.5. All acetyl polyamines tested, except acetylputrescine and acetylcadaverine, were oxidized by mPAO, although enzyme affinity for the different substrates appeared low, as determined from apparent K_m and V_{max} values

Table 1. Kinetics constants for polyamine and acetyl polyamine oxidation by pure cell wall PAO from maize shoots

Substrate	K_m (μM)	V_{max} (μmol H_2O_2 μg protein^{-1} min^{-1})
Spm	38	6
Spd	40	70
N^1-acetylSm	62	21
N^1-acetylSpd	274	2.5
N^8-acetylSpm	1130	1.5

calculated from Lineweaver–Burk plots. As shown in Table 1, only N^1-acetylSpm was a good substrate for mPAO, while N^1- and N^8-acetylSpd were oxidized slowly. These results suggest that the occurrence of an iminogroup and three positive charges on the substrate molecule are essential requisites to be a good substrate for mPAO. mPAO acts at the secondary aminogroup of polyamines [3]. The reaction products of Spd, Spm, N^8-acetylSpd and N^1-acetylSpm were analysed by TLC. All substrates tested gave DAP upon oxidation while O_2 was reduced to H_2O_2. Stoichiometric analysis of the Spd, Spm, N^8-acetylSpd and N^1-acetylSpm oxidation yielded a molar ratio of substrate to O_2, DAP and H_2O_2 of about 1:1:1:1. On the basis of these results, the oxidation pathways of N^8-acetylSpd and N^1-acetylSpm are summarized as follows:



This indicates that the cleavage points on acetylated polyamines by cell wall PAO activity are the same as with Spd and Spm oxidation. Thus, extracellular PAO is not involved in a polyamine interconversion pathway. These results obviously do not exclude the possibility that this pathway may occur in the chloroplast [10] or in other compartments [6–9]. Moreover catalytic characteristics of pure PAO may be different from those occurring when the enzyme and its substrates are in their physiological microenvironment. During the oxidation of an excess of N^1-acetylSpm, the enzyme was fast inactivated by catalysis, while the reaction with an excess of Spm or Spd proceeded at constant rate until complete O_2 depletion. The amount of N^1-acetylSpm oxidized was proportional to the amount of the enzyme added, this being inactivated after 700 enymatic cycles. After dialysis, enzyme activity was restored. A loss of enzymatic activity during polyamine degradation was previously observed for barley [13] and oat enzyme [14]. Moreover the aminoaldehydes arising from Spm and Spd degradation were demonstrated to be competitive inhibitors of mPAO [5]. In this context, we tested the effects of reaction products derived from N^1-acetylSpm oxidation on mPAO activity. Addition of DAP or H_2O_2 alone, or in combination, gave no inhibition, while the aminoaldehyde derived from N^1-acetylSpm oxidation acts as competitive inhibitor of mPAO with an apparent K_i of 2×10^{-5} M. These results suggest that the enzyme inactivation observed during N^1-acetylSpm oxidation is not due to reaction products (inactivation vs competitive inhibition) and remains unexplained. Moreover the non-competitive inhibitory activity previously reported for N^1-acetylSpm [5] is related to the rapid inactivation by catalysis of the enzyme.

EXPERIMENTAL

Chemicals. N^1-acetylSpm, N^1-acetylSpd, N^8-acetylSpd, Spm, Spd, DAP were from Sigma. All other chemicals were obtained as pure commercial products.

Enzyme purification and assay. PAO was purified as previously described [15] from shoots of 10-day-old *Zea mays* seedlings grown at 25° in the dark. Enzymatic activity was estimated polarographically according to ref. [11]. pH optimum was determined in 0.5 M KPi buffer in the pH range 3.5–8.

Identification of PAO reaction products. The enzymatic reaction was carried out in a final vol. of 1 ml containing 0.2 M NaPi pH 6.5, 1 mM appropriate polyamine, 80 nkat catalase, 50 nkat purified PAO. Incubation was performed at 37° for 10 min and stopped by addition of 200 μl, 20% (w/v) $HClO_4$. Pptd proteins were removed by centrifugation, and the supernatants were dansylated and analysed by silica gel TLC according to ref. [16].

Determination of reaction stoichiometry. The amounts of O_2 consumed and H_2O_2 formed from Spd, Spm, N^1-acetylSpm and N^8-acetylSpd by the oxidation with PAO were measured as described in ref. [17].

Reaction products of N^1*-acetylSpm oxidation.* N^1-acetylSpm (10 μmol) was incubated with 4 μkat PAO and 2 μkat catalase, both bound to hydroxyapatite in 5 ml 0.02 M KPi buffer, pH 6. Incubation was performed for 10 min at 37° under air. The reaction was stopped separating the enzyme bound to hydroxylapatite from the reaction products by centrifugation at 500 *g* for 5 min. The supernatant which was free of N^1-acetylSpm was immediately used for inhibition assays.

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