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1,3-DIAMINOPROPANE IS A SUICIDE SUBSTRATE FOR PEA DIAMINE OXIDASE

HOWLADER M. A. AWAL and EIJI HIRASAWA*

Department of Biology, Faculty of Science, Osaka City University, Osaka 558, Japan

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Key Word Index—*Pisum sativum*; Leguminosae; pea; diamine oxidase; 1,3-diaminopropane; 3-aminopropionaldehyde.

Abstract—Pea diamine oxidase (DAO, EC 1.4.3.6) oxidized 1,3-diaminopropane (1,3-DAP), but successive oxidation was suppressed during the reaction. The enzyme activity was lost after incubation with 1,3-DAP. 3-Aminopropionaldehyde (3-APA), to which DAO should oxidize 1,3-DAP, inhibited the enzyme activity.

INTRODUCTION

Putrescine (PUT) and cadaverine (CAD) are the most active substrates for the DAO from *Pisum sativum*. DAO has been shown to catalyse the oxidative deamination of a wide range of amines containing the $-CH_2NH_2$ grouping, but it was thought that 1,3-DAP is not a substrate [1]. DAOs containing copper have been found from several species of Leguminosae, and other dicots [2, 3]. On the other hand, polyamine oxidase (PAO) from *Hordeum vulgare* (EC 1.5.3.3) oxidizes spermidine and spermine to 1-pyrroline and 1-(3-aminopropyl)-pyrroline, respectively, with the additional formation in each case of 1, 3-DAP [4]. PAOs containing FAD are widely distributed in the Gramineae [5–7] and other monocots [8]. Recently, DAOs in several species of Gramineae [9–11] and a PAO in *Medicago sativa* [12] have been found. The present paper shows that 1,3-DAP is a suicide substrate for DAO.

RESULTS AND DISCUSSION

Oxidation of various amines by pea DAO

Many reports suggested that 1,3-DAP is not attacked by various amine oxidases in *Pisum sativum* [1], *Hordeum vulgare* [10], *Micrococcus rubens* [13] and *Aspergillus niger* [14], but we have now demonstrated the activity of pea DAO with 1,3-DAP as substrate, using the quinoneimine dye (QD) method [15, 16] (Fig. 1). Pea DAO unequivocally oxidized 1,3-DAP at *ca* 0.6% of the role with PUT for 5 min. However, the successive oxidation of 1,3-DAP was suppressed during the reaction. 1-Aminobutane, 1-aminopropane and 1,2-DAP that were active substrates to a small extent, were oxidized linearly during the time of reaction.

Effects of preincubation of amines and aldehydes on pea DAO activity

We examined the cause of the non-linear oxidation of 1,3-DAP using *o*-aminobenzaldehyde (*o*-ABA) method with PUT as substrate [17] (Table 1). In the case of non-preincubation, 1,3-DAP had little effect on DAO activity,

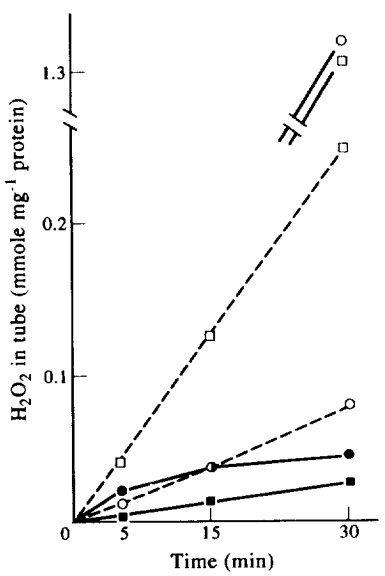

Fig. 1. Oxidation of various amines by pea diamine oxidase during time of reaction. (—○—): PUT, (– –□– –): CAD, (- - - □- - -): 1-aminopropane, (- - -○- - -): 1-aminobutane, (—●—): 1,3-DAP, (—■—): 1, 2-DAP. Details are given in the text.

*Author to whom correspondence should be addressed.

Table 1. Effects of preincubation of amines and aldehydes on pea DAO activity

Compounds in preincubations (concentrations during preincubation)	Activity (%)		
	Preincubation time		
	0 min	5 min	1 hr
None	100	100	98
1,3-Diaminopropane			
(0.1 mM)	99	79	5
(1.0 mM)	99	30	1
1-Aminopropane			
(1.0 mM)	100	91	83
3-Aminopropionaldehyde			
(0.01 mM)	90	8	4
(0.1 mM)	95	0	0
Propionaldehyde			
(1 mM)	100	98	82

Enzyme soln (0.2 ml, 24 μg) in 50 mM K-Pi buffer (pH 7) was preincubated with a compound at concentration shown in parentheses at 37° for 0 min, 5 min or 1 hr. 3-Aminopropionaldehyde was prepared from 3-aminopropionaldehyde diethylacetal soln that had been heated with 0.1 M HCl in a plugged test tube with boiled water for 10 min. Enzyme activity in 0.1 ml of the preincubated enzyme soln was estimated by *o*-ABA method with PUT as substrate. Details are given in the text.

however, little DAO activity remained after the preincubation for 1 hr with 1,3-DAP. 3-APA inhibited DAO activity after preincubation for 5 min. These results suggest that a small amount of 1,3-DAP was oxidized to 3-APA by pea DAO, and that this product inhibited DAO activity. 1,3-DAP is, therefore, a suicide substrate for DAO. Recent reports showed that DAO in pea tissue is localized exclusively in the cell wall [18]. 1,3-DAP and DAO were found in barley [10, 19]. 1,3-DAP and 3-APA may prove to be useful as a system for investigating a physiological role for DAO in cell wall in plants.

EXPERIMENTAL

Plant. Pea (*Pisum sativum* cv Alaska) seeds were germinated and grown for 8 days in moist vermiculite in plastic trays at 25° in total darkness.

Chemicals. The following were used: PUT (Sigma); 3-APA diethylacetal (Tokyo Kasei); hydroxyapatite, P-cellulose, and other chemicals as pure grade (Wako).

DAO activity. Determined according to the QD method [15, 16] or the *o*-ABA method [17]. For QD assay, the mixt. consisted of 0.5 ml of 0.1 M amines, 1 ml of 0.1 M K-Pi buffer (pH 7), 0.1 ml each of 10 mM 4-aminoantipyrine, 10 mM 3,5-dichloro-2-hydroxybenzenesulphonate and horseradish peroxidase (1 mg ml^{-1}) and appropriate amounts of the enzyme soln in a total vol. of 3 ml. The reaction was initiated by adding the amines. Incubation was 5, 15 and 30 min at 37° and the A_{515} was estimated. For *o*-ABA assay, the mixt. was composed of suitable amounts of the enzyme soln, 1 ml of 0.1 M K-Pi buffer (pH 7), 0.2 ml of 0.2% of *o*-ABA (in EtOH) and 0.5 ml of 0.1 M PUT, plus H_2O. Total vol. was 3 ml. Reaction was for 15 min at 37°. The reaction was stopped by the addition of 0.1 ml of 50% TCA. After centrifugation, A_{435} was estimated.

Pea DAO. The fraction from the first P-cellulose column chromatography obtained from *ca* 350 g of fresh pea epicotyl [20] was dialysed against 2 l of 20 mM K-Pi buffer, pH 7, overnight. The dialysed sample (30 ml) was applied to a hydroxyapatite column (1.5 × 2 cm) preequilibrated with 20 mM K-Pi buffer, pH 7. After washing the column with 100 ml of 0.1 M K-Pi buffer, pH 7, the enzyme was eluted with 0.2 M K-Pi buffer, pH 7. The active fractions were used as the pea DAO.

Protein. Determined according to ref. [12] with BSA as the standard.

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