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ALDRIN EPOXIDATION BY PLANT ROOT EXTRACTS

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Abstract—Aldrin epoxidase activity of the cell-free pea and bean root preparations was located in the particulate fraction. High speed centrifugation at 250 000 *g* for 2 hr resulted in a pellet with almost all the activity of the crude cell-free preparations. While the epoxidase was stimulated by NADPH generating system in these cell-free root preparations, that in high speed centrifugation pellets was not. Aldrin epoxidase activity of the dwarf bean root homogenates was increased by addition of *p*-aminobenzoic acid (10^{-4} M). This increase in activity is above that already manifested by Polyclar AT. No activity was detected in the dormant or germinating Alaska peas or dwarf beans until the 5th and 6th day, respectively.

INTRODUCTION

EPOXIDATION of aldrin to dieldrin by pea root preparations was first reported by Lichtenstein and Corbett[1] and the predominant activity was found in the soluble fraction. Subsequently, at least three other reports have appeared on the subject.[2-4] Aldrin epoxidase was also demonstrated in dwarf bean root extracts,[4] but predominant activity was located in the microsomal as compared to the soluble fraction obtained by differential centrifugation of root homogenates. Oloffs[3] presented evidence that aldrin epoxidase in pea roots was extracellular. The enzyme was obtained by rigorously shaking the roots in cold buffer and this preparation had no broken cells. Absence of cell organelles in this was presented as evidence that the enzyme was indeed extracellular.

The aldrin epoxidase activity in the root homogenates was considerably improved by using polyvinylpyrrolidone.[2] The activity was located in the particulate fractions of several plant roots tested.[2] Aldrin epoxidase activity in the homogenates[1] and in the cell-free preparations[3] was not stimulated by addition of NADPH. These reports point out that the enzyme is soluble[1] and extracellular.[3] Some stimulation of aldrin epoxidase was reported in dwarf bean root homogenates[4] and pea and bean root preparations[2] by the addition of NADPH.

This report presents some additional work with the homogenates and cell-free preparation of plant roots. Onset of aldrin epoxidase activity in the germinating seeds was also investigated.

RESULTS

Localization of Aldrin Epoxidase Activity in the Cell-Free Root Preparations

The cell-free root preparations of Alaska pea and dwarf beans were centrifuged at 1900 *g* for 30 min.[3] Supernatants were centrifuged at speeds indicated in Table 1 to obtain various

[1] LICHTENSTEIN, E. P. and CORBETT, J. R. (1969) *J. Agr. Food Chem.* **17**, 589.
[2] MEHENDALE, H. M., SKRENTNY, R. F. and DOROUGH, H. W. (1972) *J. Agr. Food Chem.* **20**, 398.
[3] OLOFFS, P. C. (1970) *Pesticide Sci.* **1**, 228.
[4] YU, S. J., KIIGEMAGI, U. and TERRIER, L. C. (1971) *J. Agr. Food Chem.* **19**, 5.

fractions. All the fractions were analyzed for aldrin epoxidase activity. Addition of NADPH stimulated conversion of aldrin to dieldrin by the crude extracts from both the sources (Table 1). Increasing the NADPH to 4 μmol further stimulated aldrin epoxidase and a further increase to 8 μmol resulted in an inhibition of the reaction. Upon centrifugation at high speed, most of the activity was located in the 105 000 *g* pellet. Epoxidation of aldrin to dieldrin by this fraction, however, did not seem to be stimulated by the addition of NADPH. Centrifugation at 250 000 *g* for 2 hr resulted in a supernatant with only 6·5 and 5·0% of the total activity in peas and beans, respectively. Over 93% of the activity was located in the 250 000 *g* pellet and this was not stimulated by NADPH addition.

TABLE 1. ALDRIN EPOXIDATION BY ALASKA PEA AND DWARF BEAN ROOT CELL-FREE EXTRACTS*

Fraction	nmol of dieldrin formed by 5 *g* tissue equivalents/4 hr Peas	Beans	Fraction	nmol of dieldrin formed by 5 g tissue equivalents/4 hr Peas	Beans
Crude extract	30·6	21·9	105 000 *g* pellet + NADPH	24·3	18·3
Crude extract + NADPH†	36·9	30·1	105 000 *g* supernatant	6·4	4·7
Crude extract + 2 × NADPH	44·8	39·4	105 000 *g* solubles + NADPH	7·2	4·7
Crude extract + 4 × NADPH	23·4	16·4	250 000 *g* pellet	26·7	19·4
105 000 *g* pellet	23·8	18·2	250 000 *g* soluble	2·1	1·1

* Prepared according to Oloffs.[3] † NADPH generating system equivalent to 2 μmol of NADPH.

Effect of NADPH on Dwarf Bean Crude Root Homogenate

Crude homogenates from dwarf bean roots were incubated for 8 hr in order to evaluate the effect of added NADPH at various times during incubation (Table 2). NADPH generating system was added at hourly intervals from 0 to 7 hr to the reaction flasks. Results again show that NADPH did stimulate production of dieldrin by bean crude root homogenates. Dieldrin production was maximum if NADPH was added at the time of incubation. Added NADPH had little effect if it was added after 7 hr.

TABLE 2. EFFECT OF ADDED NADPH ON ALDRIN EPOXIDATION BY DWARF BEAN ROOT CRUDE HOMOGENATE*

Time of NADPH addition after incubation (hr)	nmol of dieldrin/g tissue	Time of NADPH addition after incubation (hr)	nmol of dieldrin/g tissue
0	44·80	5	35·24
1	40·43	6	24·48
2	37·60	7	32·12
3	36·80	No NADPH	30·41
4	37·22		

* Homogenized with 0·25 g Polyclar AT per g of tissue.

Effect of PABA

p-Aminobenzoic acid (PABA) was included in the sodium phosphate buffer to give the indicated concentration (Table 3). Polyphenol oxidase activity is very widely reported in

the plants[5] and is released during homogenization and products of this reaction might interfere with aldrin epoxidation. Polyvinylpyrrolidone, a compound known to bind phenolic products, was indeed beneficial in increasing the aldrin epoxidase activity of plant root preparations.[2] PABA was included in the reactions with the hope of selectively inhibiting polyphenol oxidase. When PABA concentration was increased from 0 to 10^{-3} M, dieldrin formation was increased up to 10^{-4} M, and at 10^{-3} M PABA was inhibitory to aldrin epoxidase.

TABLE 3. EFFECT OF *P*-AMINOBENZOIC ACID ON ALDRIN EPOXIDATION BY DWARF BEAN ROOT CRUDE HOMOGENATE*

PABA (M)	Dieldrin formed nmol/g tissue/4 hr	PABA (M)	Dieldrin formed nmol/g tissue/4 hr
10^{-3}	43·23	10^{-6}	39·47
10^{-4}	51·89	10^{-7}	38·98
10^{-5}	48·54	0	39·01

* Homogenized with 0·25 g Polyclar AT per g of tissue.

Aldrin Epoxidase in the Seed Germinates

Presoaked Alaska peas and dwarf beans were allowed to germinate on wet filter paper discs. Seeds were kept moist by covering with wet filter paper discs. Germinating seeds were removed at 16, 40, 64, 88 and 132 hr. Seeds along with the hypocotyls were homogenized and the crude homogenates were incubated with aldrin and heptachlor. Aldrin epoxidase activity was not detected in peas or beans allowed to germinate up to 88 hr. At 132 hr 7·5 and 4·1 nmol of dieldrin was produced by peas and beans, respectively, per standard incubation flask. Heptachlor was not epoxidized by any of the above seed homogenates.

DISCUSSION

Aldrin epoxidase activity of the cell-free pea root preparations is predominantly attributable to the particulate fraction. When extracts were centrifuged at 250 000 *g*, almost all of the activity was located in the pellet. The activity might be associated with the root wall debri, which come off during the rigorous shaking in buffer that is required to extract the enzyme. At any rate, the enzyme is particulate and is not soluble. Addition of NADPH to the cell-free root preparations did stimulate dieldrin production. Addition of excess NADPH generating system, however, inhibited the reactions.

Added NADPH does stimulate dieldrin production in dwarf bean root homogenates also. Increased dieldrin formation is maximum if the cofactor is added at the time of incubation. There is a possibility that epoxidation of aldrin takes place due to two or more enzymes. This is especially evident if one considers the results in Table 2. Adding NADPH to the reaction at the time of incubation has maximum beneficial effect than adding it after any length of incubation. This might mean that the enzyme system capable of oxidizing aldrin with the utilization of NADPH becomes progressively inactive after incubation at 37°.

Aldrin epoxidation was not stimulated by addition of NADPH to high centrifugation fractions. Microsomal preparations were consistently non-responsive to NADPH generating

[5] GILLELTE, J. R. (1963) *Progr. Drug. Res* **6**, 11.

system. Mg^{2+} ions are necessary with NADPH for oxidative metabolism of other compounds by liver microsomes[5] and insect fat bodies.[6] Failure of the microsomal aldrin epoxidase to respond to added NADPH generating system is not due to lack of Mg^{2+} since inclusion of Mg^{2+} inhibited the reaction. This observation suggests the presence of two or more enzyme systems in the root homogenates which are capable of epoxidising aldrin.

Aldrin epoxidase activity in the plant root extracts may be further increased by utilizing the right amount of PABA. It is not clear as to how PABA might be causing this increase. One possibility seems to be by indirect protection of the epoxidase activity by preventing the accumulation of inhibitory phenolic products which usually result due to polyphenol oxidase activity in plants extracts. Although direct protection of the enzyme activity by PABA cannot be ruled out, this seems unlikely since the increase in activity is caused only by a narrow range of its concentration (10^{-4}–10^{-5} M). While lower concentrations are ineffective, higher concentration is inhibitory. The increased activity in dwarf bean root homogenate imparted by 10^{-4} to 10^{-5} M PABA is over and above that brought about by the use of Polyclar AT.

Aldrin epoxidase activity is not present in the dormant or germinating seeds of Alaska peas or dwarf beans. The activity appears only on 5th and 6th days for peas and beans, respectively. Hypocotyl of peas was about 13 mm or longer and about 6·5 mm in beans when the onset of epoxidase activity occurs. Maximum enzyme activity was reported to be between 9 and 21 days in case of dwarf beans, and beyond 21 days the activity declined.[4]

Highest aldrin epoxidase activity reported for cell-free root preparations is about 2·45% dieldrin per g of pea roots.[3] Root homogenates with Polyclar AT give about 3·84% dieldrin per g of pea roots.[2] From the point of obtaining most active preparations, homogenization seems to be the choice.

EXPERIMENTAL

Aldrin, dieldrin, heptachlor and heptachlor epoxide were obtained from Analabs, Inc., North Haven, Connecticut. Analytical grade reagents and pesticide quality solvents were used. Polyclar AT (polyvinylpyrrolidone) was supplied gratis by GAF Corporation, New York, N.Y.

Plant materials used in the study were raised and active root preparations were made as reported earlier.[2] Seeds presoaked for 26 hr were planted in vermiculite wooden flats and watered as necessary and maintained in a greenhouse. Two- to three-week-old plants were used in the study. Roots were homogenized with indicated amount of Polyclar AT, using cold sodium phosphate (0·1 M, 6·5 pH) buffer. The homogenate was filtered through 4 layers of cheesecloth and the filtrate termed as crude homogenate. Cell-free root preparations were made according to Oloffs.[3] 105 000 *g* and 250 000 *g* pellets were obtained by centrifuging 1900 *g* supernatant of this cell-free preparation for 2 hr at those speeds. Standard 5 ml incubation mixture included 1 g equivalents of tissue homogenates, or 5 g equivalents of cell-free preparations, sodium phosphate (0·1 M, 6·5 pH) buffer, 2 μmol NADP, 20 μmol glucose-6-phosphate, and 1 unit of glucose-6-phosphate dehydrogenase. Reactions were started by adding 400 μg of aldrin in 20 μl EtOH using a microsyringe. Incubations were made at 37° for 4 hr unless otherwise indicated.

The reactions were stopped by adding 4 ml hexane–isopropanol (3:2) and stored at −20° until extracted. Extractions were carried out in glass-stoppered centrifuge tubes using hexane. Recoveries of above 95% were obtained using this procedure for extraction.[2] The extracts were analyzed on Varian Aerograph Model 1800 GLC with EC detection under the following operating conditions: standard metal 3·3 mm × 180 cm columns packed with either DC200 or SE30 on Anachrom ABS (80/90 mesh) were used. Injector port, 200°; column, 180°; detector, 210° and nitrogen carrier gas, 42 ml per min.

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[6] CHAKRABARTHY, J., SISSONS, C. H. and SMITH, J. N. (1967) *Biochem. J.* **102,** 492.