The Depression of the Synthesis of Pea Diamine Oxidase due to Light and the Verification of its Participation in Growth Processes using Competitive Inhibitors

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Abstract. The time courses of the synthesis of diamine oxidase in pea plants grown for 14 days either in the light or in the dark are similar with the highest increase in activity occurring in the cotyledons and in the shoots during the first 6 to 8 days. Plants grown in the dark showed a 2- to 3-fold higher enzyme activity than plants grown in the light. Pea diamine oxidase could be in vivo efficiently inhibited by substrate analogues 1,4-diamino-2-butanono and 1,5-diamino-3-pentanone. The first compound inhibited proportionally to its concentration the growth of etiolated pea plants, but its instability makes an unequivocal interpretation of the results difficult. On the other hand, 1,5-diamino-3-pentanono a stable and more efficient diamine oxidase inhibitor depressed the growth of pea seedlings only at concentrations as high as 5 mM and 10 mM, at which the growth of cress seedlings not containing diamine oxidase was also strongly depressed. The results obtained indicate that tryptamine oxidation catalyzed by diamine oxidase is not involved in the main metabolic pathway leading from tryptophan to indoleacetate in pea plants.

Young pea plants contain high diamine oxidase activity [diamine: oxygen oxidoreductase (deaminating), EC 1.4.3.6.] which is sometimes simply referred to as amine oxidase. The synthesis of the enzyme starts in the first hours of germination and in normal green plants reaches the highest activity after the first few days (Kenter and Mann 1952, Werle et al. 1959, 1961). Because amine oxidases are able to oxidize tryptamine, some authors supposed that it could be involved in the formation of indoleacetate (Kenter and Mann 1952, Clarke and Mann 1957, Lantican and Muir 1967). Experiments carried out so far mostly with the inhibitors of hydrazine type lead, however, to contradictory conclusions. Reed et al. (1965) and recently Kimura and Tanaka (1971) found a relation between depressed growth of pea plants and the inhibition of tryptamine oxidation caused by the herbicide B-995 or by monoamine oxidase inhibitors safrazine and nialamide. On the
contrary, R. Moss from the Gordon's laboratory (see LARSEN 1967, page 433) found that indoleacacetate production was not changed in the pea stem tissues in which tryptamine oxidase activity was nearly completely inhibited. Aminoguanidine, isonicotinic acid hydrazide, and isopropylhydrazine inactivating diamine oxidase activity in vivo inhibited the germination not only in pea but even in the plant species not containing this enzyme (WERLE et al. 1959). In this paper we therefore try to verify if the selective inhibition of diamine oxidase can influence the germination and growth of pea seedlings. Nearly nontoxic substrate analogues 1,4-diamo-2-butane and 1,5-diamo-3-pentanone were used as efficient competitive inhibitors of diamine oxidase; they do not affect mitochondrial monoamine oxidase (MACHOLÁN et al. 1967, MACHOLÁN 1974). Another aim of this paper was to follow the dynamics of the formation of diamine oxidase in pea plants grown either in the light or in the dark.

Material and Methods

1,4-diamo-2-butane dihydrochloride was prepared after MICHÁLSKÝ et al. (1953) and 1,5-diamo-3-pentanone was synthesized according to a method devised in our laboratory (MACHOLÁN 1974).

Cultivation of the Plants

The experiments were performed with pea seedlings (Pisum sativum L.) cv. Liliput, and, cress plants (Lepidium sativum L.) were used for bioassays. Selected seeds were surface sterilized for 5 min. with 96% ethanol, rinsed with water, and then soaked for 24 h in distilled water or in inhibitor solution of pH approx. 5.0. The seeds were then transferred onto a layer of granulated polyethylene underlayered with diluted (1:4) Richtor's nutrient solution supplemented with microelements in Hoagland A-Z solution. The germination and further cultivation occurred at 23°C either in the dark or in continuous light (white and rosy fluorescent tubes 1:1, 7 000 lx at the level of the plants) in a chamber with controlled conditions. The volume of the nutrient solution was kept constant by replenishing with distilled water. In the experiments with the inhibitors, groups of 250 seedlings, 3 to 4 days old, were transferred into 1000 ml flat plastic cultivation vessels containing 500 ml of diluted (1:1) nutrient solution either with the inhibitor or without it.

Enzyme Extraction and Assays

10 to 22 pea seedlings were carefully washed, and then cotyledons, roots, and shoots were separated and weighted. Testa were removed from the cotyledons and each plant part was separately homogenized in a mortar with chilled 0.1 M phosphate buffer pH 7.0 and silica sand (0.2 g of sand per g of plant material). The homogenate was squeezed through a nylon cloth and the remaining pellet was repeatedly extracted with the buffer solution. The filtrate was made up to 50 or 100 ml with the buffer solution and immediately assayed.

Protein was determined in the supernatant after centrifuging the homogenate at 10 000 g for 15 min using a modified biuret method with the subtraction of the turbidity after the addition of KCN (SZARKOWSKA and KLEINGENBERG 1963). Bovine serum albumin was used as standard protein.

Diamine oxidase activity was determined spectrophotometrically (HOLMSTEDT et al. 1961, MACHOLÁN 1966) at 25°C. The assay mixture (final volume 5 ml) consisted of 50 mM phosphate buffer pH 7.0, 2.5 mM o-aminobenzaldehyde, 20 μg catalase, 10 mM 1,4-diaminobutane, and 0.5 ml plant extract. One unit (U) represents the amount of enzyme catalyzing the formation of one μmole Δ1-pyrroline per min. Specific activity is expressed in units per mg protein.

Results

Diamine Oxidase Level in Pea Plants Grown Either in the Light or in the Dark

Diamine oxidase activity was followed in extracts from different parts of plants grown in continuous light or in the dark during the first 14 days. The