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SHORT REPORTS

INHIBITOR OF DIAMINE OXIDASE IN COTYLEDONS OF GROUNDNUT SEEDLINGS

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Key Word Index—*Arachis hypogea*; Leguminosae; groundnut; diamine oxidase; diamine oxidase inhibitor.

Abstract—The apparent absence of diamine oxidase in extracts of cotyledon of germinating groundnut seeds is due to the presence of a natural inhibitor. The inhibitor was associated with the lipid in the upper layer obtained on centrifugation of the cotyledon extract. It was non-dialysable, thermolabile and was inactivated by TCA and Triton X-100. The inhibitor was detected in the cotyledon extracts from early stages of seed development and was present up to 20 days after germination.

INTRODUCTION

Amine oxidases, which may be involved in the regulation of intracellular polyamine concentration, are widely distributed [1]. Polyamines stimulate growth in several microbial and tissue culture systems probably because of their interaction with nucleic acids [2]. Moreover, increased levels of spermidine have been reported in rapidly growing cells [3]. The possibility that the amine oxidase influences the growth rate by regulating the polyamine levels makes the study of this enzyme of considerable interest. The pea diamine oxidase (DAO) has a broad specificity and oxidizes many primary amines [4]. It has been shown [5] that the pea enzyme, which may have a role in the formation of IAA, is regulated in a feedback manner by auxins and is induced by its substrates. Recently, it has been reported [6] that the pea cotyledon DAO is also induced by phytic acid, which suggests that the levels of polyamines may be controlled by this compound. The apparent absence of DAO in groundnut cotyledons observed in the present study is due to the presence of an inhibitor and in this communication, we report some properties of this inhibitor.

RESULTS AND DISCUSSION

Inhibitory effect of cotyledon extract

DAO activity was not detected in developing or resting groundnut seeds but during germination it appeared in embryo on day 2 (3.5 nkat/g fr. wt), increased up to day 4 (5.6 nkat/g fr. wt) and remained constant thereafter up to 12 days (5–5.6 nkat/g fr. wt). The cotyledons, however, did not show the DAO activity during this period of germination when extracts were prepared in Pi buffer. This apparent absence of DAO activity was due to the presence of an inhibitor in the cotyledon since addition of the groundnut cotyledon extract to pea cotyledon or groundnut embryo DAO resulted in *ca* 70% inhibition. The inhibitor was localized in the upper lipid layer obtained on centrifugation of the cotyledon extract at 10 000 g for 30 min. The extent of DAO inhibition increased by adding increasing concentrations of lipid layer to the assay. At higher concentrations of lipid layer the DAO activity could be completely abolished.

Properties of inhibitor

The inhibitor was non-dialysable, thermolabile and was inactivated by TCA treatment. These studies indicated that the inhibitor may be a protein and since it was associated with the lipid layer it was thought to be a lipid protein complex. This was confirmed by extracting the lipids in organic solvents. Exhaustive extraction of the lipid layer by $CHCl_3$, MeOH and Me_2CO resulted in the loss of inhibitory activity. To investigate whether both lipid and protein components of the inhibitor are essential, the lipid layer was treated with Triton X-100 for different intervals in cold. Triton, which is known to break lipid–protein complexes, completely abolished the inhibitory activity of the lipid layer within 20 min.

Reconstitution of inhibitor

To investigate whether the inhibitor, after removal of the associated lipid, can again be reconstituted, the groundnut oil was mixed with the proteins obtained after Me_2CO extraction. The mixture so obtained was able to inhibit the DAO to the same extent as the

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untreated cotyledon extract. The groundnut oil or the protein alone did not show any inhibition. The groundnut protein could also not be replaced by bovine serum albumin.

Presence of inhibitor during development and germination

To investigate the presence of inhibitor during development of seeds, groundnut plants were obtained from the field, and seeds were separated into different groups on the basis of seed wt. The average fr. wt/seed and the inhibitory activity of each group containing 40–50 seeds were determined. The inhibitor was present at early stages of development, and it increased *ca* 2-fold when the seed reached maturity. The activity of the inhibitor was reduced to *ca* 45% in the cotyledon after 20 days of germination.

Demonstration of DAO activity in cotyledons after removal of inhibitor

Triton X-100 treatment of the lipid layer of the cotyledon extract prevented the inhibition and the extract showed DAO activity if prepared in Pi-buffered (10 mM, pH 7) Triton (0.25%) instead of Pi buffer alone. The DAO was detected on day 4 (0.2 nkat/g fr. wt) and increased with age. After 6, 8 and 12 days germination, DAO activity was 0.2, 0.3 and 0.4 nkat/g fr. wt, respectively. Similar results were obtained when the extracts were prepared in Pi buffer and centrifuged. The inhibitor was removed in the lipid layer and the aqueous layer was used for the enzyme assay.

These studies thus show that groundnut cotyledon extracts have DAO which is not detected in the assay due to the presence of an inhibitor. Though at the moment it is difficult to assign a specific role to this inhibitor, it is tempting to speculate that it may be involved in the regulation of polyamine concentration by controlling DAO activity.

EXPERIMENTAL

Plant material. Groundnut seeds (*Arachis hypogea* L. cv Punjab-1) obtained from a local farm were soaked and germinated as described in ref. [7]. The time when seeds were kept for germination, after soaking for 16 hr, was considered as day zero of germination.

Enzyme and inhibitor extracts. Pea and groundnut cotyledon extracts were prepared by grinding the tissue with 10 mM Pi buffer, pH 7 in a chilled pestle-mortar at 0°. A 10% (w/v) extract was prepared. In some studies Pi buffer was replaced by Triton X-100 buffered with 10 mM Pi buffer, pH 7. DAO activity was determined according to the method of ref. [8]. The assay system consisted of 50 μmol Pi buffer, pH 7.5; 5 μmol putrescine, 0.1 μmol pyridoxal phosphate and enzyme (0.2 ml) in a total vol. of 4 ml. After incubation at 37° for 30 min, the reaction was terminated by adding 0.5 ml of 10% TCA followed by 0.1 ml (10 mg/ml) *o*-aminobenzaldehyde. The A_{430} nm of the complex was measured, after removal of proteins by centrifugation ($E_0 = 1.86 \times 10^3$/mol/cm [8]). One unit of inhibitor is the amount of inhibitor required to completely inhibit one unit of DAO. Experiments using Warburg manometry indicated that the inhibitor did not interfere with the formation of the pyrroline/*o*-aminobenzaldehyde complex.

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