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Protection by GABA and Succinic Semialdehyde of Seed Germination and Some Enzymatic Activities Against High Concentration of Hydroxylamine

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Hydroxylamine was found to stimulate germination of *Lupinus albus* at concentrations inferior to 10 mM and to inhibit it greatly at 20 mM concentration. This inhibition was partially restored by GABA or succinic semialdehyde. Hydroxylamine, at high concentrations, behaved as inhibitor *in vivo* on GABA 2-oxyglutarate aminotransferase and succinic semialdehyde dehydrogenase NAD-dependent, whereas it behaved as activator on succinic semialdehyde dehydrogenase NADP-dependent. No effects were observed on the enzymatic activities and the inhibited germination was partially restored, after GABA and succinic semialdehyde had been added to a growth medium with a 20 mM hydroxylamine concentration. A possible protection mechanism of GABA and succinic semialdehyde against hydroxylamine action is discussed.

The enzymes conected with formation and utilization of GABA have been shown to occur in plants (1, 4, 6) and all their enzymatic activities are greatly increased during germination of *Lupinus albus* seeds (1). This seems to indicate that some metabolites of the GABA pathway could be implicated in the seeds germination. In order to check this it was decided to inhibit some enzymes of this pathway. Hydroxylamine, a well known inhibitor of aminotransferases, was used Several concentrations of it were employed and its effect on germination and GABA aminotransferase and succinic semialdehyde dehydrogenase were checked.

Materials and Methods

Plant material. *Lupinus albus* seeds from Sevilla (Spain) recolected on August 1973 were germinated on vermiculite moistened with distilled water or different concentrations of hydroxylamine used as hydroxylamine-HCl at pH 7 with sodium hydroxyde. Succinic semialdehyde was

prepared according to PRESCOTT *et al.* (7). Germination was carried out at 25° C under constant illumination. In order to keep the humidity degree constant, the seeds were sprinkled, each day, with 10 ml of the suitable solutions. Emergence of the radicle was the adopte criterion of germination. The hydroxylamine was prepared before using and then kept in cold at 4° C during germination time (about 10-15 days).

Preparation of cell free extracts. Complete seeds, embryos or cotyledons were grounded in a mortar with 10 volumes of. 0.05 M of Tris-HCl buffer pH 7.9 at 2-4° C. The extracts were stirring during 1 minute, then, were filtered through cheescloth and centrifuged at 25,000 × *g* for 30 minutes. Supernatant was used as enzymatic solution.

Enzymatic assays. GABA aminotransferase was assayed according to ANDRÉS *et al.* (1).

Succinic semialdehyde dehydrogenase was followed by measuring the disappearance of succinic semialdehyde in the reaction according to BESSMAN *et al.* (2). The incubation medium contained 200 μmoles of potassium phosphate buffer pH 7.1, 300 μmoles of succinic semialdehyde; 25 μmoles of 2-mercaptoethanol 1.5 μmoles of NAD or NADP and 0.5 ml of enzymatic solution, in a total volume of 1 ml. Incubations were carried out for 30 minutes at 37° C. GABA aminotransferase as well as succinic semialdehyde dehydrogenase reactions were stopped by adding 1 ml of 20 % trichloroacetid acid.

Enzymatic activities are expresed as ΔOD/mg of protein. Protein concentration was determined by LOWRY *et al.* (5).

Results

Germination of *Lupinus albus* seeds in water started after four days and greatly increased up to ten days. Afterwards the

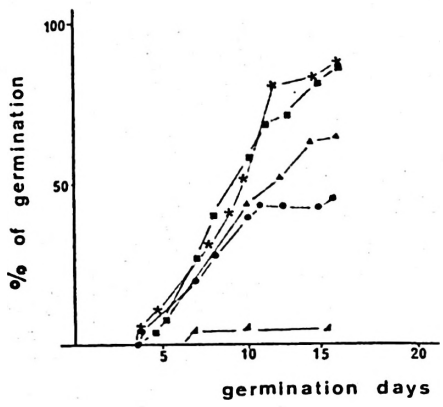

Fig. 1. *Effect of different hydroxylamine concentrations on germination of* Lupinus albus *seeds.*
● Control (water); ✱ 1 mM NH_2OH; ■ 5 mM NH_2OH; ▲ 10 mM NH_2OH and ◢ 20 mM NH_2OH.

Table I. *Effect* in vivo *of hydroxylamine and hydroxylamine plus succinic semialdehyde on GABA aminotransferase from* Lupinus albus *seeds.*
The seeds were germinated in the presence of water (Control) or the indicated solutions. Enzymatic activities were determined in the whole seeds after 4 days of germination.

Conditions	Specific activity	% I
Control	7.3	0.0
+ 10 mM NH_2OH	3.1	58.0
+ 10 mM NH_2OH + 0.5 mM Succinic semialdehyde	4.9	32.0

Table II. *Effect* in vivo *of hydroxylamine and hydroxylamine plus GABA on GABA aminotransferase activity from* Lupinus albus *seeds.* Germination conditions as indicated in table I.

Conditions	Specific activities	
	Embryos	Cotyledons
Control	21.3	5.7
+ 10 mM NH_2OH	11.2	0.0
+ 10 mM NH_2OH + + 10 mM GABA	21.8	4.2

Table III *Effect of hydroxylamine and hydroxylamine plus succinic semialdehyde on succinic semialdehyde dehydrogenase activity from* Lupinus albus *seeds.*
Experimental conditions as indicated en legend of table I.

Conditions	Specific activities	
	NAD-linked	NADP-linked
Control	4.6	2.1
+ 10 mM NH_2OH	3.2	7.8
+ 10 mM NH_2OH + 0.5 mM Succinic semialdehyde	5.1	3.2

Table IV. *Disappearance of NH_2OH from seeds which were germinated in the presence of hydroxylamine and hydroxylamine plus GABA.* Germination conditions as indicated in table I. Hydroxylamine was measured according to TIHAMER *et al.* (9).

Conditions	μMoles of free hydroxylamine	
	Embryos	Cotyledons
Control	0.0	0.0
+ 20 mM NH_2OH	240.0	320.0
+ 20 mM NH_2OH + + 20 mM GABA	80.0	130.0

development followed at least for 15 days. Addition of hydroxylamine to the growth mixture increased the germination at concentrations between 1-10 mM but at 20 mM a great inhibition was observed and at higher concentrations (100 mM) germination did not take place (fig. 1). These results agree with those of HENDRICKS *et al.* (3) which found that hydroxylamine promoted germination of several seeds at 0.1 mM or less concentrations.

The effect of hydroxylamine on enzymatic activities responsible of the GABA pathway was tested *in vivo* on whole seed and on embryos and cotyledons. Hydroxylamine at concentration 10 mM greatly inhibited the specific activity of GABA aminotransferase (table I and II)

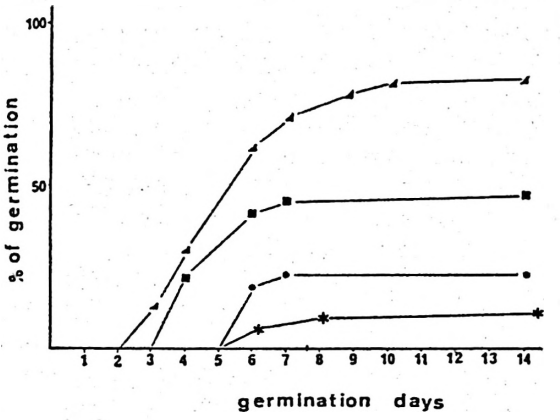

Fig. 2. *Effect of GABA and succinic semialdehyde on germination of* Lupinus albus *seeds grown in the presence of high concentrations of hydroxylamine.*
◢ Control (water); ✱ 20 mM NH_2OH; ■ 20 mM NH_2OH plus 80 mM GABA; ● 20 mM NH_2OH plus 8 mM of succinic semialdehyde.

and succinic semialdehyde dehydrogenase NAD-linked while actived the NADP-linked (table III). At this concentration germination was not inhibited but stimulated (fig. 2). Addition of succinic semialdehyde (about 0.5 mM) in the presence of 10 mM of hydroxylamine restored GABA aminotransferase activity in a 25 % (table I) and reversed the effect on succinic semialdehyde dehydrogenase (table III). GABA addition at the concentration of 10 mM, completely restored the GABA aminotransferase activity from embryos and, in a great extent, in cotyledons (table II). The addition of succinic semialdehyde or GABA to the growth medium with 20 mM of hydroxylamine restored germination (fig. 2). The concentration of free hydroxylamine disappear from seeds in the presence of GABA (table IV).

Discussion

From the results presented in this study, it is clear that hydroxylamine salts promotes germination at 10 mM or less con-

centrations but inhibits GABA aminotransferase and succinic semialdehyde dehydrogenase NAD-linked while actives the NADP-linked enzyme. The differences in behavior of two succinic semialdehyde dehydrogenases against hydroxylamine action suggest, as it is the case for *Pseudomones* (6), the presence, in plants, of two succinic semialdehyde dehydrogenases with different specificity by the coenzyme.

Inhibition of GABA aminotransferase by 10 mM of hydroxylamine did not implicate a decrease in germination, what suggest, that succinic semialdehyde and GABA were not directly relationated with germination.

The inhibitor effect of great concentrations of hydroxylamine on germination could be due at its action on oxygen consumption. Hydroxylamine is a strong reductant and a strong chelating agent. It reacts to form oximes with aldehydes and ketones, or nitrogen ethers with aldehydes (8). In a metabolic sense it can be reduced to NH_2X and can probably form an oxime with the formyl group of *cytochrome a* to limit electron flow to oxygen or associated phosphorylation (4) in respiration. Formation of oximes, followed by reduction, has sometimes been considered in amino acid formation (10, 11). As hydroxylamine can form oximes with aldehydes and ketones it is very probable that the mechanism of protection of GABA and succinic semialdehyde be due to the formation of oximes with both agents. The fact of the disappearance of hydroxylamine in the presence of GABA (table IV) could prove that.

The main conclusion that it is possible to claim from this study is that GABA and succinic semialdehyde protect the seeds germination and some enzymatic activities against high concentrations of hydroxylamine.

Resumen

La hidroxilamina a concentraciones inferiores de 10 mM estimula la germinación de las semillas de *Lupinus albus* y la inhibe a concentraciones mayores. Esta inhibición se restaura, parcialmente, en presencia de GABA o semialdehído succínico. La hidroxilamina 10 mM es inhibidora *in vivo* tanto de la GABA aminotransferasa como de la semialdehído succínico dehidrogenasa NAD-dependiente, pero activa al enzima NADP-dependiente. Si al medio de crecimiento con hidroxilamina se le adiciona GABA o semialdehído succínico no se aprecia efecto sobre dichas actividades enzimáticas y se restaura, en parte, la germinación inhibida por la hidroxilamina. Se discute un posible mecanismo de protección del GABA y semialdehído succínico frente a la acción *in vivo* de la hidroxilamina.

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