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EFFECT OF SELENIUM ON CHLOROPHYLL BIOSYNTHESIS IN MUNG BEAN SEEDLINGS

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Key Word Index—*Phaseolus vulgaris*; Leguminosae; mung bean; 5-aminolevulinic acid; chlorophyll; PBG-synthase; selenium.

Abstract—The effect of selenium on chlorophyll biosynthesis was investigated in mung bean seedlings, both dark-grown as well as grown under natural photoperiods. Selenium had no effect on the synthesis of 5-aminolevulinic acid, but repressed the levels of protochlorophyllide and chlorophyll in dark-grown seedlings. Selenium inhibited porphobilinogen synthase activity and decreased total chlorophyll content in light grown seedlings. The dose-dependent response of porphobilinogen synthase activity and chlorophyll content to selenium suggest the possible role of this enzyme in chlorophyll biosynthesis. The inhibition of porphobilinogen synthase activity in isolated chloroplasts *in vitro* by selenium further confirmed *in vivo* inhibition of porphobilinogen synthase and the importance of this enzyme in chlorophyll biosynthesis.

INTRODUCTION

Early surveys [1, 2] established the association of at least two dozen species of *Astragalus* with seleniferous soils, which were divided into accumulator and non-accumulator plants. Trelease and Trelease [3] have grown the accumulator and non-accumulator species of *Astragalus* in liquid and sand in the presence of selenite. Interestingly they observed augmented growth in the cultures of accumulators and retarded growth in the case of non-accumulators. Both these species were found to have different selenium assimilation patterns. In addition to *Astragalus* species, other plants also synthesize organic derivatives from selenium [4]. Most of the selenium compounds have as their counterparts sulphur containing compounds which are known metabolites in plants and micro-organisms [4, 5]. These facts and other findings, suggest the interference of selenium compounds with sulphur metabolism by a typical anti-metabolite action.

Earlier studies indicated selenium as a micronutrient for several animals [6, 7] but not in plants [8]. However, recent studies report that there is growth stimulation by selenium and its role is confirmed as a micronutrient in species other than *Astragalus* [9]. Though many studies were carried out on selenium, most of the work was concentrated on the nutritional aspects and metabolism of selenium in plant systems. No reports are available at present on the effect of this element on other vital process of plants such as chlorophyll biosynthesis.

Our recent studies and those of others show that porphobilinogen synthase (PBG-synthase), a metal sensitive enzyme is found to play a major role in the regulation of chlorophyll synthesis [10–12]. Hence, in the present studies an attempt has been made to see the effect of selenium on chlorophyll synthesis and the role of PBG-synthase in the regulation of chlorophyll synthesis.

RESULTS AND DISCUSSION

Germinating seedlings of mung bean were treated with different concentrations of selenium (12.5, 25.0 and 62.5 μM). The seedlings were removed at 24 hr intervals and PBG-synthase activity and chlorophyll contents were estimated. The effect of selenium on PBG-synthase activity is shown in Fig. 1. The treatment of seedlings with selenium inhibited the PBG-synthase activity and there was nearly 50% of inhibition at 62.5 μM concentration of

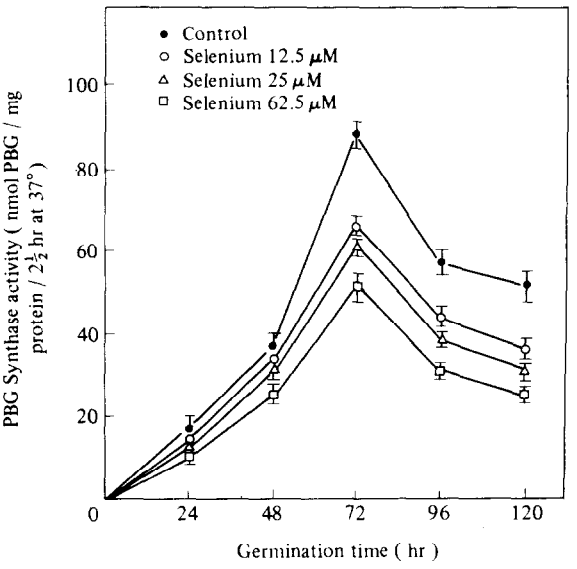

Fig. 1. Effect of selenium on PBG-synthase activity in germinating seedlings of mung bean.

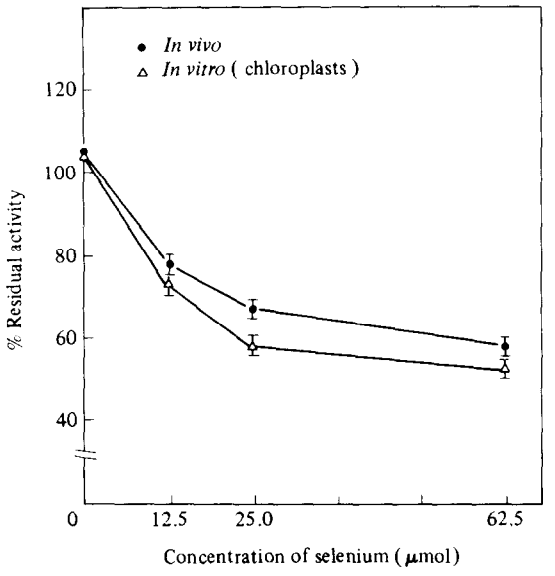

Fig. 2. *In vivo* effect of selenium on PBG-synthase activity in four-day-old seedlings of mung bean (●—●) and *in vitro* effect of selenium on PBG-synthase activity in isolated chloroplasts (△—△). Chloroplasts were isolated from four-day-old seedlings and ruptured by sonication. Selenium (12.5, 25.0 and 62.5 μm) was added to the reaction mixture and the activity was assayed as described in the text.

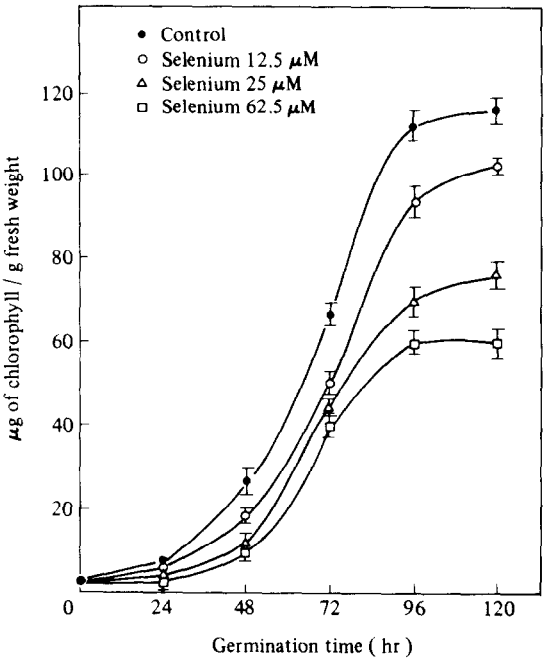

Fig. 3. Effect of selenium on chlorophyll synthesis in germinating seedlings of mung bean.

selenium. In both control and treated seedlings, the PBG-synthase activity increased with age up to the third day and decreased on consequent days. Transformation of plastids into chloroplasts was reflected by the increase in PBG-synthase activity with age. As PBG-synthase was exclusively located in chloroplasts fraction [11, 12], the effect of selenium on chloroplastic PBG-synthase has been studied. Selenium inhibited the PBG-synthase activity in chloroplasts isolated from four-day-old seedlings *in vitro*. The enzyme activity was reduced to half at 62.5 μM selenium (Fig. 2). This shows that the inhibition of PBG-synthase activity was similar both *in vivo* and *in vitro* in four-day-old seedlings of mung bean.

Our results also showed that chlorophyll levels increased upto the fourth day of germination and remained constant on day five (Fig. 3). Further, it was observed that increasing concentrations of selenium decreased the total chlorophyll content. This indicates the complete maturation of the chloroplasts by day four of germination under the conditions employed.

Our earlier work [11, 12] on the effect of lead and mercury on chlorophyll synthesis in both *in vivo* and *in vitro* revealed that 5-aminolevulinic acid (ALA) synthetic ability was unaltered by these metals whereas ALA dehydratase (PBG-synthase) activity was inhibited. The effect of selenium on chlorophyll synthesis is essentially similar to that of lead and mercury but different from the inhibition of chlorophyll biosynthesis by cadmium [13], where ALA synthetic ability was also inhibited by cadmium.

Selenium showed no effect on the synthesis of ALA but decreased the total chlorophyll content in dark-grown seedlings, both in the presence and absence of levulinic acid (Table 1). Levulinic acid is a competitive inhibitor of PBG-synthase and under these conditions ALA accumulates. The chlorophyll and ALA in the leaves were determined after the final light treatment. To examine whether selenium was an effective inhibitor of any of the steps between ALA and chlorophyll synthesis, the effect of selenium on protochlorophyllide (Pchld) content was studied. Selenium depressed the Pchld content in dark-grown seedlings by nearly 50% (Table 2). This observation indicates the interaction of selenium with chlorophyll biosynthesis prior to Pchld formation, probably at the level of PBG-synthase and other enzymes.

The biosynthesis of tetrapyrroles proceeds through a metal sensitive enzyme PBG-synthase, which requires the presence of thiols regardless of the source [14]. Our results show that increasing selenium concentrations correspondingly decreased the PBG formation by inhibiting the PBG-synthase activity, which was required for chlorophyll biosynthesis. Further, the *in vitro* incubation of the enzyme with various concentrations of selenium showed that selenium has significant effect on PBG-synthase in micromolar concentrations. Based on the *in vitro* studies of PBG-synthase by selenium, the inhibition can be attributed to the oxidation of free -SH groups present at the active sites of the enzyme. Selenite and selenious acid (formed by the reaction between SeO_2 and H_2O) were found to oxidize thiol groups [15, 16]. Bergstermann [17] demonstrated the inhibition of succinate dehydrogenase, a thiol group containing enzyme from various sources by selenite. Moreover, the inhibition of selenite was reversed by the addition of cysteine. However, the exact mechanism of selenium interaction with PBG-synthase is yet to be established.

Table 1. Effect of selenium on the production of 5-aminolevulinic acid and chlorophyll

Treatment	ALA (nmol/g fr. wt)	Chlorophyll (μg/g fr. wt of leaves)
Distilled water only	380±30	445
Distilled water + levulinic acid	590±60	16
Selenium 12.5 μM	400±20	390
Selenium 12.5 μM + levulinic acid	600±20	12
Selenium 25.0 μM	375±40	340
Selenium 25.0 μM + levulinic acid	590±30	10
Selenium 62.5 μM	380±40	280
Selenium 62.5 μM + levulinic acid	580±20	9

*Leaves from four-day-old dark-grown seedlings were treated with selenium for 10 hr in the dark and transferred to a solution of levulinic acid (50 mM) for a further 1 hr in the dark. The leaves were exposed to light for 7 hr after which chlorophyll and ALA were assayed. Samples of 0.5 g were taken for estimations.

Table 2. Effect of selenium on protochlorophyllide levels in dark-grown mung bean seedlings

Treatment	Protochlorophyllide	
	nmol/g fr. wt.	% Reduction
Distilled water only	90.8±10	—
Selenium 12.5 μM	66.0±15	27
Selenium 25.0 μM	57.0±15	37
Selenium 62.5 μM	45.8±10	50

*Treatment and other details are as in Table 1 except for the levulinic acid treatment.

EXPERIMENTAL

Mung beans (*Phaseolus vulgaris* L.) were germinated and grown on a moist filter paper in petri dishes under natural day light at day and night temps of 30 and 26°, respectively. The controls were maintained with H_2O and other lots of seedlings were treated with different concns. (12.5, 25.0 and 62.5 μM) of selenium solns (added as SeO_2), PBG-synthase activity and chlorophyll levels were estimated at regular intervals. Another batch of seedlings were grown in complete darkness and maintained with H_2O for 4 days. The primary leaves were cut and treated with different concns of selenium soln for 10 hr in the dark and then were exposed to light (2.80 W/m^{-2}) for 7 hr. Another set of leaves were treated with levulinic acid (50 mM) for 1 hr in the dark before they were transferred for light treatment. ALA, Pchld and chlorophyll contents were estimated after final light treatment. All manipulations of the dark-grown material were carried out under a dim-green safe light in the dark.

ALA was estimated by the method of Mauzerall and Granick [18]. ALA from leaves was extracted in 10% trichloroacetic acid and condensed with Ac_2O in the presence of 2 M NaOAc buffer (pH 4.6). Modified Ehrlich reagent was added to the condensate (1:1) and the colour developed was read at 553 nm. ALA levels were expressed as nmol/g fr. wt.

Crude extract of PBG-synthase was prepared by homogenization of seedlings with 0.05 M Tris–HCl buffer (pH 8.2) containing 0.1 M dithiothreitol. The homogenate was filtered through 4 layers of muslin cloth and centrifuged for 15 min at 15000 *g* at 4°. The supernatant was used as an enzyme source. PBG-synthase activity was assayed according to the method of Schneider [19]. Enzyme extract (1.0 ml) was incubated with 0.27 ml 1 mg/ml ALA, 1.35 ml 0.05 M Tris–HCl buffer (pH 8.2) with 0.1 M dithiothreitol and 0.08 ml 0.02 M $MgCl_2$ for 2.5 hr at 37°. After incubation the reaction was stopped with 0.3 ml 3 M trichloroacetic acid and centrifuged at 2000 *g* for 10 min. Modified Ehrlich reagent was added to the supernatant (1:1) and the Ehrlich chromophore [18] was measured at 553 nm after 15 min. Protein was estimated by method of ref. [20], using BSA as a standard. Chlorophyll content was estimated spectrophotometrically by the method of ref. [21]. The Pchld content was measured by the alkaline acetone extraction method [22] using the BASIC computer program for the analysis of absorbance data. Chloroplasts were isolated from four-day-old seedlings by the method of ref. [23].

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