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# EFFECT OF LEAD AND MERCURY ON CHLOROPHYLL SYNTHESIS IN MUNG BEAN SEEDLINGS

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**Key Word Index**—Mung bean (*Phaseolus vulgaris*, L.); Leguminosae; δ-aminolevulinic acid dehydratase; heavy metals; porphobilinogen; chlorophyll.

**Abstract**—Germinating seeds of mung bean were treated with different concentrations of lead and mercury. Estimations of δ-aminolevulinic acid dehydratase activity, chlorophyll and protein contents were performed. The metals were found to inhibit δ-aminolevulinic acid dehydratase activity and decrease total chlorophyll content, suggesting the possible regulatory role of the enzyme in chlorophyll synthesis. The δ-aminolevulinic acid dehydratase activity was localized exclusively in chloroplasts.

## INTRODUCTION

It is a common observation that elements not known to be essential to plants are nevertheless accumulated by plants in appreciable quantities. The accumulation of heavy metals by plants from contaminated soils and nutrient solutions results in impaired metabolism and retarded growth [1–4]. Further, the relationship between the number of vehicles/unit of time and their speed to the lead content in plants has been well established [5]. There are also reports that the photosynthesis of higher plants is highly sensitive to metals like cadmium and lead [6, 7]. The formation of δ-aminolevulinic acid is the first identified step in tetrapyrrole biosynthesis leading to haem, chlorophyll, vitamin $B_{12}$ and other specialized linear tetrapyrroles found in plants [8]. Aminolevulinic acid synthetase, a pyridoxal phosphate requiring enzyme [9] found in bacteria [10, 11], yeast [12] and higher animal tissues [13, 14], has been ascribed a central role in the regulation of chlorophyll synthesis. Recent reports show that δ-aminolevulinic acid dehydratase (ALAD), a metal-sensitive enzyme [15], is found to regulate chlorophyll synthesis [16].

The present studies were designed to elucidate the possible mechanism of lead and mercury on chlorophyll biosynthesis at the level of δ-aminolevulinic acid dehydratase, the second rate limiting enzyme in tetrapyrrole biosynthesis.

## RESULTS AND DISCUSSION

Germinating seedlings of mung bean were treated with different concentrations of metal solutions (50, 100 and 250 μM) of lead and mercury, respectively. The seedlings were removed at regular intervals and ALAD activity and chlorophyll estimations were performed. Treatment of seedlings with lead and mercury (250 μM) inhibited the ALAD activity by 50%. In both control and treated seedlings ALAD activity was increased with growth up to the 4th day and decreased on the 5th day (Figs 1 and 2). The increase in ALAD activity with age reflects the growth and transformation of plastids into chloroplasts, which follows chlorophyll synthesis. The results showed that chlorophyll levels increased up to the 4th day of germination and remained constant on day 5. Further, it was observed that the total chlorophyll content was dose-dependent and decreased with increasing metal concentration (Figs 3 and 4). This indicates that the chloroplasts become almost fully developed on the 4th day of germination under the conditions employed. However, interestingly the levels of aminolevulinic acid, which is the substrate for ALAD, were unaltered by lead and mercury. Hence, our results show that chlorophyll synthesis was altered by lead and mercury interacting at both ALAD activity and chlorophyll levels. Earlier reports on the inhibitory effect of lead on ALAD in isolated chloroplasts of spinach leaves [15] and chlorophyll levels of etiolated leaves of *Avena sativa* were found to be similar [17].

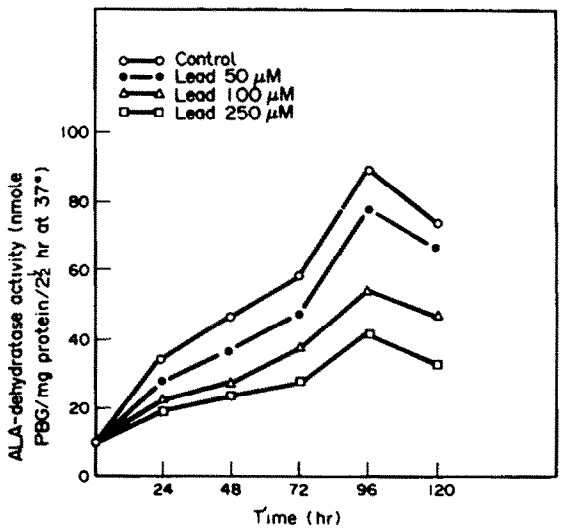


Fig. 1. Effect of lead on δ-ALA-dehydratase activity in germinating seedlings of mung bean.

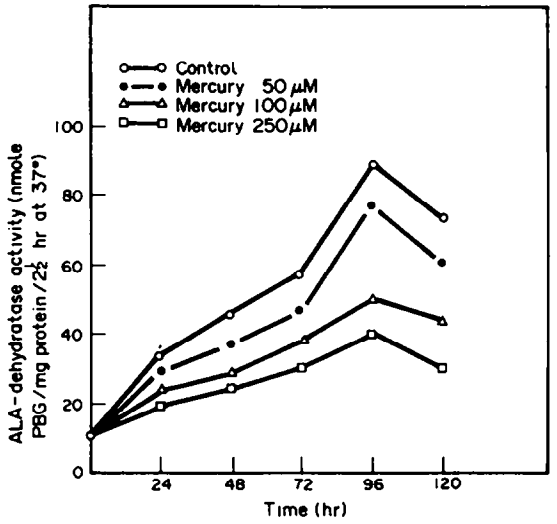


Fig. 2. Effect of mercury on δ-ALA-dehydratase activity in germinating seedlings of mung bean.

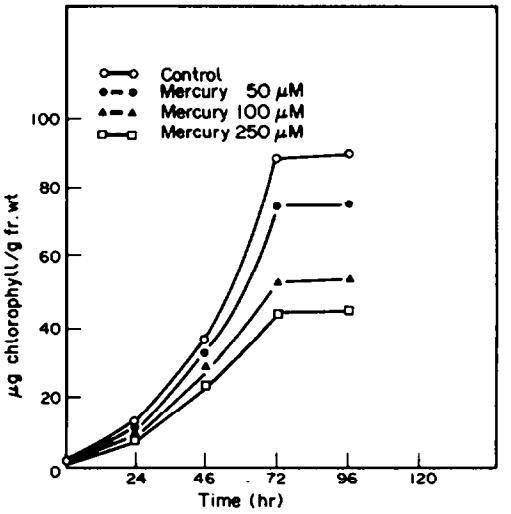


Fig. 4. Effect of mercury on chlorophyll synthesis in germinating seedlings of mung bean.

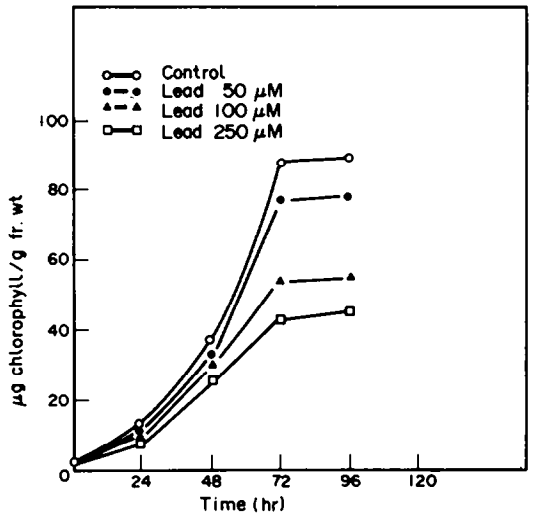


Fig. 3. Effect of lead on chlorophyll synthesis in germinating seedlings of mung bean.

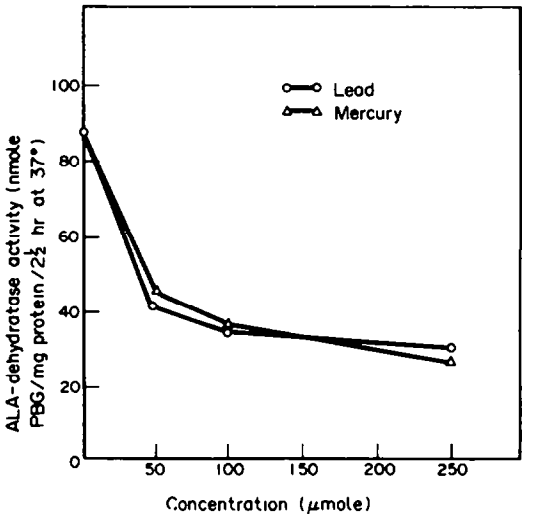


Fig. 5. *In vitro* effect of lead and mercury on ALA-dehydratase in 4-day-old seedlings of mung bean. Buffer extracts of the enzyme from 4-day-old seedlings were preincubated with different concentrations of metal solutions (50 μM, 100 μM and 250 μM) of lead and mercury for 30 min at 37°. Aliquots were taken after incubation and enzyme activity was assayed as described in the text with proper controls.

The biosynthesis of tetrapyrroles proceeds through a metal sensitive enzyme, ALAD, which requires thiol activation regardless of the source [18]. Our results show that increasing metal concentrations correspondingly decreased the formation of porphobilinogen by inhibiting the ALAD activity, which was required for chlorophyll synthesis. The decreased activity may be due to the interaction of the heavy metals with –SH groups of the enzyme at active sites and the inhibition of ALAD by heavy metals was found to be of the uncompetitive type [19]. This was further confirmed by *in vitro* incubation of ALAD with lead and mercury resulting in decreased activity (Fig. 5), indicating the possible interaction of these metals with thiol groups.

Subcellular distribution studies indicate that the enzyme is exclusively localized in chloroplasts and no activity was observed in mitochondria and cytosol fractions. Previous data on the localization of ALAD in greening leaves indicate that ALAD is exclusively present in chloroplasts and etioplasts [20]. In tobacco [21] and cucumber [22] ALAD was found to be a stromal enzyme and the enzyme localization was said to be species specific. Our results suggest that this enzyme is not membrane-bound as we could detect much less activity in intact chloroplasts and there was an increased activity when the isolated chloroplasts were ruptured with a hypotonic

medium. The release of ALAD into the lysate suggests that the enzyme might be in soluble form in stroma or loosely bound to lamella.

The mode of action of lead and mercury was found to be similar on ALAD activity and chlorophyll synthesis. In the present findings, there is strong evidence for a possible regulatory role of ALAD on chlorophyll synthesis in both untreated and treated seedlings.

## EXPERIMENTAL

Mung beans (*Phaseolus vulgaris*, L.) were germinated and grown on a moist filter paper in Petri dishes under natural daylight at day and night temperatures of 30 and 26°. The controls were maintained with dist. $H_2O$ and other lots of seeds were treated separately with metal solns of different concns (50, 100 and 250 $\mu$M) of lead and mercury [added as $(CH_3COO)_2 \cdot Pb \cdot 3H_2O$ and $HgCl_2$]. Seedlings were removed at regular intervals to estimate ALAD activity and chlorophyll content.

Crude enzyme was prepared by homogenization of seedlings in 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 15 000 *g* at 4°. The supernatant was used as an enzyme source. ALAD activity was determined by assaying the PBG formation [23]. 1 ml of enzyme was incubated with 0.27 ml of 1 mg/ml ALA; 1.35 ml of 0.05 M Tris–HCl buffer (pH 8.2) with 0.1 M dithiothreitol and 0.08 ml of 0.02 M $MgCl_2$ for 2.5 hr at 37°. After incubation the reaction was stopped with 0.3 ml of 3 M trichloroacetic acid and centrifuged at 2000 *g* for 15 min. To the supernatant Ehrlich reagent (1 : 1) was added [24] and the optical densities were read at 555 nm after 15 min. Enzyme activity was expressed as nmol of PBG formed/mg protein/2.5 hr at 37°. Protein estimations were done according to the method of ref. [25], using BSA as a standard.

Chlorophyll content was measured spectrophotometrically by the method of ref. [26].

Chloroplasts and mitochondria were isolated from 4-day-old seedlings of mung bean. Chloroplasts were isolated by the method of ref. [27] and ruptured by osmotic shock with hypotonic medium. Mitochondria were isolated according to the method of ref. [28] and were lysed osmotically.

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