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BRIEF COMMUNICATION

Inhibition of chlorophyll biosynthesis by lead in greening *Pisum sativum* leaf segments

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Abstract

Supply of 0.01 to 1.0 mM lead acetate to greening pea (*Pisum sativum* L.) leaf segments either in the absence or in the presence of inorganic nitrogen lowered total chlorophyll (Chl) content. During a time course study, there was not any appreciable effect of Pb^{2+} upto 4 h but thereafter Pb inhibited Chl synthesis. While supply of succinate, cysteine dithiothreitol, 5,5-dithio-bis-2-nitrobenzoic acid and NH_4Cl had no protective action against Pb^{2+} toxicity, and glycine, glutamate 2-oxoglutarate, $MgCl_2$, KH_2PO_4, $CaCl_2$, KCl protected only partially, reduced glutathione (GSH) could completely overcome the inhibition of Chl biosynthesis by the metal. It is suggested that Pb^{2+} interferes with Chl biosynthesis through GSH availability.

Additional key words: glutathione, nitrogen, pea, pollution, protective agents, thiol compounds.

Lead is a metallic pollutant emanating in the environment from various sources including industrial wastes combustion of fossil fuels and use of agro-chemicals. It adversly affects plant life (Broyer *et al.* 1972, Merakchiiska *et al.* 1976). Lead inhibits Chl synthesis in soybean (Bazzaz *et al.* 1974), *Platanus occidentalis* (Carlson and Bazzaz 1977), maize (Bazzaz *et al.* 1975, Carlson *et al.* 1975), loblolly pine, autumn olive (Rolfe and Bazzaz 1979) pea and maize (Sinha 1988a, b). The present investigation was undertaken to study the effect of Pb^{2+} on Chl synthesis in etiolated leaf segments, with a goal to gain some insight into the possible mechanism.

Seeds of *Pisum sativum* L. purchased from National Seeds Corporation, New Delhi, were surface sterilised with 0.1 % bleaching powder ($CaOCl_2$) for about 5 min and then washed thoroughly with distilled water. The seedlings were raised in plastic pots filled with acid washed sand, in continous darkness for 12 d at 25 ± 2 °C.

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They were irrigated on alternate days with 1/2 strength Hoagland's solution (pH 6.0) containg no nitrogen. For various treatments primary leaves from uniformly grown seedlings were cut into about 2 mm wide segments and floated on 1/2 strength Hoagland's solution containing either no nitrogen or 10 mM KNO_3 or 10 mM NH_4Cl as sole nitrogen source, and lead as desired. The samples were incubated for the desired period at irradiance of about 65 W m^{-2} and temperature 27 ± 2 °C.

Chl was extracted from the treated samples with 80 % acetone in cold and its quantity was calculated from absorbance at 645 and 663 mm according to Strain and Svec (1966). The results presented are average values of at least three independent experiments ± S.E.

Supply of 0.01, 0.1 and 1.0 mM lead acetate to excised pea leaf segments from etiolated seedlings lowered Chl biosynthesis both in absence as well as presence of inorganic nitrogen in the form of KNO_3 or NH_4Cl (Table 1). Generally, the negative effect of Pb increased with its concentration. When excised leaf segments were incubated in Hoagland's solution ($+NO_3$) in the light, the total Chl content increased almost linearly after 4 h upto 24 h (Fig. 1). Further incubation upto 48 h caused a little increase in the Chl content. In the presence of lead the Chl content was low, although it increased slightly upto 24 h.

Table 1. Effect of lead supply either in absence (-N) or presence of inorganic nitrogen on Chl content in greening pea leaf segments from 12-d-old dark grown seedlings floating on 1/2 strength Hoagland's solution with the shown supplements for 24 h under continuous irradiance 65 Wm^{-2} at 27 ± 2 °C. Values relative to control are given in parentheses.

Pb^{2+} [mM]	Chl (a+b) [mg kg^{-1}(f.m.)]		
	-N	+10 mM KNO_3	+10 mM NH_4Cl
0.0 (control)	288.2 ± 8.2 (100)	296.2 ± 10.2 (100)	268.2 ± 6.4 (100)
0.01	244.7 ± 6.4 (95)	264.4 ± 12.2 (89)	225.0 ± 5.3 (84)
0.1	185.5 ± 6.8 (64)	154.2 ± 14.7 (52)	160.0 ± 6.8 (60)
1.0	54.4 ± 7.2 (19)	60.4 ± 6.4 (20)	61.4 ± 5.3 (23)

Some precursor of Chl biosynthesis, glutamate, 2-oxoglutarate, $MgCl_2$, KH_2PO_4, $CaCl_2$, KCl and NH_4Cl, when supplied alongwith lead, partially reversed the inhibitory effect of the metal on Chl biosynthesis (Table 2). On the other hand, glycine and succinate had no effect on inhibition. From the tested thiol compounds cysteine (5 mM), 5,5-dithio-bis-2-nitro benzoic acid (DTNB) and dithiothreitol (DTT) [0.1 mM each] had no effect but the supply of reduced glutathione (5 mM) completely reversed the inhibitory effect of lead on Chl biosynthesis (Table 2).

The inhibitory effect of Pb^{2+} on Chl biosynthesis in leaf segments was independent of inorganic nitrogen assimilation. Also the effect of inorganic nitrogen supply itself on Chl biosynthesis during a 24 h treatment was marginal (Table 1). There was also a lag period in the inhibition observed, which might account for the time taken for uptake and transfer of heavy metal to the site of its action. The

experiments also indicate that the site of inhibition was at the level of availability of 2-oxoglutarate and glutamate for the synthesis of 5-aminolevulinic acid.

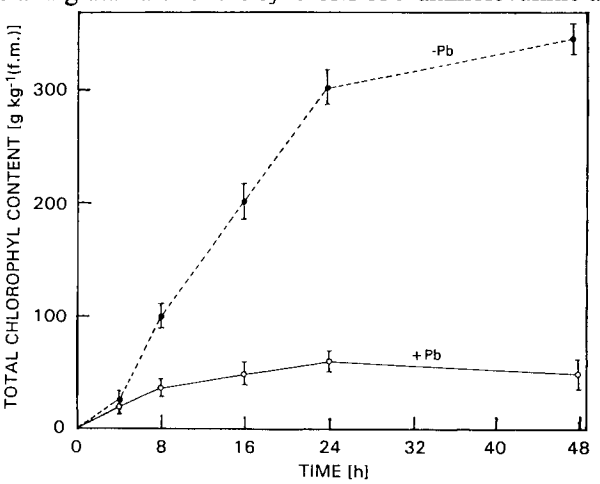

Fig. 1. Time course of increase in total chlorophyll content in greening pea leaf segments in the absence or presence of lead. Leaf segments from 12-d-old dark grown seedlings were floated on 1/2 strength Hoagland's solution containing 10 mM KNO_3 either in the absence or presence of 0.1 mM Pb^{2+} under continuous irradiance 65 Wm^{-2} at 27 ± 2 °C.

Table 2. Effect of some precursors of chlorophyll (Chl) biosynthesis and thiol compounds on the inhibition of Chl biosynthesis by lead in greening maize leaf segments leaf segments from 12-d old dark grown seedling were floated on 1/2 strength Hoagland's solution in the presence of 0.1 mM Pb^{2+} and desired precursor compound for 24 h under irradiance 65 Wm^{-2} at 27 ± 2 °C.

Additive in the nutrient solution	Chl (*a*+*b*) [g kg^{-1}(f m.)]	% of control
None (control)	296.4 ± 4.8	100
Pb (0.1 mM)	172.1 ± 10.0	58
Pb + 10 mM glutamate	260.8 ± 8.2	88
Pb + 10 mM 2-oxoglutarate	262.4 ± 6.2	89
Pb + 10 mM glycine	168.0 ± 3 0	57
Pb + 10 mM succinate	167.8 ± 3.4	57
Pb + 10 mM KH_2PO_4	290.4 ± 2.6	98
Pb + 10 mM $CaCl_2$	256.4 ± 1.4	86
Pb + 10 mM $MgCl_2$	240.6 ± 2.6	81
Pb + 10 mM KCl	270.8 ± 4.2	91
Pb + 10 mM NH_4Cl	178.2 ± 3 6	60
Pb + 10 mM cysteine	170.0 ± 3.3	58
Pb + 0 1 mM DTNB	169.0 ± 2.4	57
Pb + 0.1 mM DTT	190.2 ± 1.4	64
Pb + 5 μM glutathione	298.6 ± 1 7	101

The negative effect of lead on the Chl biosynthesis could be partially counteracted by the addition of glutamate and oxoglutarate or nutrient salts KH_2PO_4, $CaCl_2$ and

KCl. Addition of NH_4Cl, however, could not replace these salts. These salts may in the presence of Pb^{2+} replace the cations and anions available for the biosynthesis of Chl (Table 2). The inhibition in Chl biosynthesis is possibility at the level of inhibition of 5-aminolevulinic acid dehydratase as an important enzyme in the biosynthesis of heme compounds including Chl (Nandi and Waygod 1967, Nandi *et al.* 1968, Burzynski and Grabowski 1984). The most interesting observation in our study was an almost complete neutralization of the inhibitory effect of lead by GSH but not other thiol compounds (Table 2). GSH is involved directly or indirectly in the synthesis of proteins, DNA transport, enzyme activity metabolism and protection of cells, *etc.* (Meister and Anderson 1983). Although its specific role in Chl biosynthesis is not clear, GSH is precusor of phytochelatin synthesis in cultured tomato cells (Scheller *et al.* 1987). Phytochelatin synthesis is induced by heavy metals and the metals are inactivated by complexing with it (Grill *et al.* 1985). Thus whatever the target site may be GHS may reverse the inhibitory effect of Pb by possible forming phytochelatins and inactivating the heavy metal itself.

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