BRIEF COMMUNICATION

Influence of lead on membrane permeability and lipoxygenase activity in lupine roots

R. RUCIŃSKA and E.A. GWÓŹDŹ*

Laboratory of Plant Ecophysiology, Faculty of Biology, Adam Mickiewicz University, Al. Niepodległości 14, PL-61713 Poznań, Poland

Abstract

Lead nitrate at concentration of 150 mg dm^{-3} inhibits root growth of *Lupinus luteus* seedlings by about 20 %, which is accompanied by an increase of K^+ leakage from the root cells. Non-denaturing isoelectric focusing in polyacrylamide slab gel has shown that lead stimulates the activity of most lipoxygenase isoenzymes and induces one additional isoenzyme with pI 6.9.

Additional key words: K^+ leakage, lipid peroxidation, *Lupinus luteus*.

Heavy metals may induce alteration in plasma membrane integrity throughout lipid peroxidation (De Vos *et al.* 1989). This process is initiated by redox active metal ions themselves, for example Cu^{2+} (Weckx and Clijsters 1996). Metal ions, unable to perform univalent oxidoreduction reaction such as Zn^{2+}, Pb^{2+}, Cd^{2+} and Hg^{2+}, stimulate the lipoxygenase (LOX, EC 1.13.11.12) activity (Weckx and Clijsters 1997, Bhattacharjee 1998, Mishra and Choudhuri 1999). This enzyme mediates the conversion of polyunsaturated fatty acids to their conjugated hydroperoxidiene derivatives using molecular oxygen (Gaillard and Chan 1980). The process of membrane lipid peroxidation generates broad range of products, including small hydrocarbon fragments, for instance malondialdehyde (MDA) (Halliwell and Gutteridge 1989). Increased concentration of MDA in metal-exposed plants have been reported (Hartley-Whitaker *et al.* 2001, Shah *et al.* 2001, Gonnelli *et al.* 2001). The modification of the plasma membrane properties causes the leakage of potassium ions (Weckx and Clijsters 1996, 1997, Shainberg *et al.* 2000).

The objective of present study was to investigate the changes in potassium leakage, lipid peroxidation and LOX activity in lupine roots exposed to Pb^{2+}. This study was designed to show a possible correlation between the alteration in the plasma membrane integrity and the activity of lipoxygenase under lead stress.

Seeds of yellow lupine (*Lupinus luteus* L. cv. Juno) were germinated in Petri dishes containing water-moistened filter paper until the roots reached 6 - 8 mm in length. Subsequently, 100 seeds were put in the holes of plastic sieve hanging on hooks in crystallising dish filled with 400 cm^3 of 1/2-strength Hoagland's medium. Incubation was carried out in the dark at 23 °C and cultures were aerated every 3 h. After 72 h the medium was removed and seedlings were transferred to water (control) or water solution of $Pb(NO_3)_2$ containing 150 mg dm^{-3} Pb^{2+}. The plants were maintained in a controlled climate room at temperature of 23 °C, relative humidity of 46 %, a 16-h photoperiod, irradiance of 150 μmol m^{-2} s^{-1} and aeration. The leakage of potassium from roots was measured either in water or in solution of 150 mg dm^{-3} Pb^{2+} (400 cm^3) after 24, 48, 72, 96 h of incubation with appropriate ion-selective electrode. Root tips (5 mm long) were homogenised in 0.2 M boric buffer pH 7.0 or 0.15 M phosphate buffer pH 7.6 and then centrifuged for 15 min at 13 000 *g*. The protein concentration in the supernatant was estimated according to Bradford (1976). Proteins isolated with boric buffer were electrophoresed in 7 % (m/v) polyacrylamide gel

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Abbreviations: LOX - lipoxygenase; MDA - malondialdehyde; TBArm - thiobarbituric acid reactive metabolites.
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* Corresponding author: fax: (+48) 61 8294518, e-mail: albus@amu.edu.pl

containing ampholytes, pH 3.5 - 10 (Jackowski and Przymusiński 1995). Isoenzymes of LOX were visualised by the method of Heinish *et al.* (1996). Lipid peroxidation was determined according to Hendry and Grime (1993) in samples homogenised with phosphate buffer. The level of lipid peroxidation was expressed as the content of thiobarbituric acid reactive metabolites (TBArm). The absorbance was measured at 532 nm minus non-specific absorbance at 600 nm by spectrophotometer UV-1202 (*Shimadzu*, Japan)

The experiments were carried out on the seedlings cultured in the presence of 150 mg dm^{-3} Pb^{2+}. This concentration of Pb^{2+} had slight effect on the fresh mass of roots, which was inhibited by 20 % regardless of the time of treatment (Table 1). At the same time significant release of K^+ (Table 1) was observed. It has been reported that lead ions increase the relative leakage (Bhattacharjee 1998) and electrical conductivity (Mishra and Choudhuri 1999) in germinated seedlings of *Amaranthus lividus* and *Oryza sativa*, respectively. Moreover, a correlation between membrane injury and enhanced lipoxygenase activity has been demonstrated as a result of lead treatment (Bhattacharjee 1998, Mishra and Choudhuri 1999).

Table 1. Fresh mass [g], potassium leakage [mg dm^{-3} g^{-1}(f.m.)] and thiobarbituric acid reactive metabolites (TBArm) [$A_{532-600}$ g^{-1}(f.m.)] in lupine roots incubated in water or 150 mg dm^{-3} $Pb(NO_3)_2$ for 24 - 96 h. Data are means ± SE of at least 3 measurements.

$Pb(NO_3)_2$	Treatment	Fresh mass	K^+ leakage	TBArm
0	24 h	18.2 ± 1.0	0.1 ± 0.006	0.53 ± 0.02
	48 h	18.4 ± 0.7	0.2 ± 0.007	0.47 ± 0.01
	72 h	17.6 ± 1.7	0.1± 0.005	0.37 ± 0.09
	96 h	18.8 ± 2.4	0.1 ± 0.005	0.37 ± 0.04
150	24 h	14.3 ± 0.7	1.4 ± 0.3	0.36 ± 0.03
	48 h	13.7 ± 2.7	2.6 ± 0.5	0.09 ± 0.01
	72 h	12.5 ± 0.5	3.3 ± 0.9	0.07 ± 0.01
	96 h	16.2 ± 2.0	3.3 ± 0.5	0.07 ± 0.01

The lipoxygenase activity in control lupine roots showed constant rise during 4 d of seedling development (Fig. 1). Four isoenzyme bands with distinct isoelectric points (pI 4.2, 4.7, 5.2 and 5.3) represented LOX from control roots (Fig. 1). Full isoenzyme pattern with one additional isoenzyme (pI 3.5) appeared after 96 h of root growth. In lead-treated roots activity of all isoenzymes increased rapidly already after 24 h. Moreover lead induced one additional isoform of pI 6.9 and the activity of this isoenzyme was time-dependent and peaked after 96 h of lead exposure (Fig. 1).

The various isoforms of lipoxygenase have been classified as two types: one with optimum activity at relatively high pH 8 - 9 (type-1) and the second one, which is the most active at near neutral pH 6 - 7 (type-2) (Siedow 1991). Type-1 uses only free fatty acids as substrates, in contrast to type-2 which oxygenates the estrified unsaturated fatty acids moieties in membranes (Maccarone *et al.* 1994). In our study the activity of LOX was estimated by staining intensity on IEF native gels at pH 7.0, which favours activity of enzymes related to type-2. It is thus likely that isoenzymes of lipoxygenase induced or activated by lead ions in lupine roots directly attack membrane fatty acids, which in turn contribute to enhanced K^+ leakage.

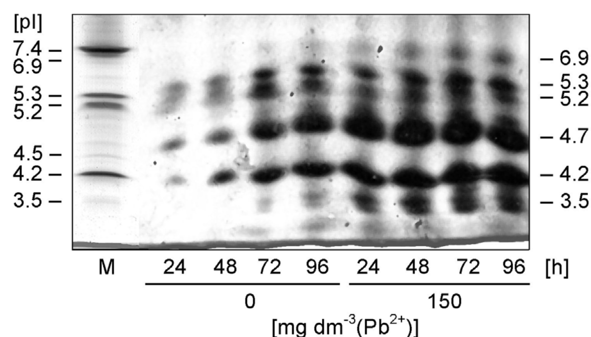

Fig. 1. Isoenzyme pattern of lipoxygenase in lupine roots incubated in water or 150 mg dm^{-3} $Pb(NO_3)_2$. Soluble proteins were electrophoresed on 7 % IEF slab gel and isoenzymes were visualised by staining with 1 % (m/v) N,N-dimethyl-*p*-phenylenediaine.

In lupine roots the content of thiobarbituric acid reactive metabolites (TBArm), which reflects the rate of lipid peroxidation, decreased already 24 h after lead application (Table 1). These data are inconsistent with the results recorded by Bhattacharjee (1998) and Mishra and Choudhuri (1999) who indicated that lead enhanced the accumulation of MDA. The low level of TBArm in lupine roots might be explained by the formation of cross-links between protein molecules and MDA, which in turn can cause severe damage to membrane proteins and electrolyte leakage from the cells (Halliwell and Gutteridge 1989). Alternatively, the hydroperoxides, resulting from the action of LOX, could be converted into more stable compounds, including traumatic or jasmonic acid, which participate in the defense reaction to biotic and abiotic stresses (Siedow 1991).

Our results suggest that peroxidative degradation of membrane lipids might be involved in the alteration of membrane permeability in lupine roots exposed to lead. It seems that the induction or the stimulation of the activity of various isoforms of lipoxygenase initiates this process. However, further experiments are needed to establish what kind of primary and secondary products of this enzyme are formed in the presence of lead ions.

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