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Effect of lead on the lipid metabolism in spinach leaves and thylakoid membranes

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Abstract

The effects of lead and sodium acetate treatment on the lipid composition of leaves, thylakoid membranes and cell debris of spinach were investigated. The concentration of lead in leaves and cell debris was higher than that in thylakoid membranes, probably due to a protection of photosynthetic apparatus. The lead treatment lead to decrease of contents of monogalactosyl diacylglycerols and phospholipids and to increase of the other glycolipids in the thylakoid membranes. There were no statistically significant differences between the total lipids of thylakoid membranes after incubation with lead and sodium acetate, which was an indication that in this case the effect of metal ion was not specific.

Keywords: digalactosyl diacylglycerols, monogalactosyl diacylglycerols, phospholipids, *Spinacia oleracea*, sulphoquinovosyl diacylglycerols, triacylglycerols

Introduction

There are numerous investigations (see reviews of Baker 1987, Ernst *et al.* 1992) on the influence of heavy metals on the plant metabolism, but the data for accumulation of lead in the leaves with their ageing are very limited (Ernst 1984).

Commonly accepted is the opinion, that plants contain specific metal-bonding proteins, similar to metallothioneins (Lolkema *et al.* 1984) and more than 90 % of heavy metal ions are located in the cell wall fraction of roots (Morishita and Boratynsky 1992). Nevertheless, a part of the heavy metal ions can penetrate into the above-ground parts of plants. The oldest leaves of metal-exposed plants generally exhibit the highest metal concentrations. Due to the low mobility of lead in the

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Abbreviations: CD - cell debris; DGDG - digalactosyl diacylglycerols; L - whole leaves; MGDG - monogalactosyl diacylglycerols; PL - phospholipids; SQDG - sulphoquinovosyl diacylglycerols; TAG - triacylglycerols; TM - thylakoid membranes.

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environment and its significant accumulation in the roots, the concentration of lead in senescent leaves remain constant throughout the year (Ernst *et al.* 1992).

Our observations on the effect of lead acetate incubation on 15-d-old seedlings of maize (Stefanov *et al.* 1993) showed that only about 45 % of the lead appeared in the roots and the remaining 55 % were in the leaves. Evidently, different plants have different ability to accumulate heavy metal ions in their roots. Some plants can remove metals via natural leaf shedding, but if leaves are used as food, like salad or spinach, the heavy metal content in the leaves may be even dangerous for health.

The effect of heavy metals on the photosynthetic apparatus (Baszynski *et al.* 1982, Shioi *et al.*1978, Hsu and Lee 1988) and on the lipid and fatty acid composition (Maskymiec *et al.* 1992) in spinach chloroplasts is well investigated *in vitro*, but little is known on the accumulation of lead in the leaves.

In this report we present data on the accumulation of lead in whole leaves, thylakoid membranes and cell debris from spinach (*Spinacia oleracea* L.) and its effect on their lipid composition.

Materials and methods

Plant material: Spinach seeds *(Spinacia oleracea* L. cv. Matador) were germinated on an inert substrate (perlite) with Hogland's nutrient solution (9.5 meq dm^{-3}). 23 d after sowing (the second leaf formation) the plants were transferred to plastic pots (1.2 dm^3), filled with nutrient solution (19 meq dm^{-3}) for one week, and then the concentration of the nutrient solution was adjusted to 38 meq dm^{-3} (31 d after sowing). Fe in chelate form in a concentration of 7.5 mg dm^{-3} and Mo in a concentration of 0.01 mg dm^{-3} were added to the nutrient solution. The nutrient solutions volume was kept constant by daily corrections; they were aerated twice a day for 20 min and changed twice a week. During the experiment, plants were grown in a greenhouse (natural light, photoperiod 14/10 h, day/night temperature 26/20 °C, air humidity 40 - 60 %).

At the age of 38 d, the plants were separated into five groups (40 plants per group). One group was kept in pure nutrient solution (control), the second and the third group were transferred to nutrient solutions, containing 3.8 and 38 mg dm^{-3} lead acetate (equivalent to 2.4 and 24 mg dm^{-3} Pb^{2+}). The fourth and fifth group of plants were grown in the same way, but with 2.8 and 28 mg dm^{-3} sodium acetate solution (the same amounts of acetate ions as in the above-mentioned solutions of lead acetate).

Samples of the leaves (30 g fresh mass each) were collected twice: 56 and 64 d after sowing.

Isolation of the thylakoid membranes: Washed leaves (100 g) were ground at 4 °C for 15 s in *Waring* blender in 375 cm^3 of a medium containing in a buffer containing 350 mM NaCl, 10 mM KH_2PO_4, 10 mM ascorbate, 3.3 mM cystein, 50 mM 2-mercaptoethanol and 50 mM Tris-HCl (pH 8.0) (Heber and Santarius 1964) The homogenate was filtered through *Miracloth* and centrifuged for 1 min at 600 *g*. The

supernatant was subjected to 1 min centrifugation at 1500 *g*. The pellet was resuspended in a medium containing 350 mM NaCl and 50 mM Tris-HCl, pH 8.0 and centrifuged for 1 min at 2000 *g*. The sedimented chloroplasts were broken with distilled water and the released thylakoid membranes (TM) were centrifuged at 2000 *g*. This washing procedure was repeated once more. The pellet from the first centrifugation and the supernatant from the second centrifugation were the cell debris (CD). The TM and CD were analyzed separately.

Lead analysis: A sample of intact leaves, TM and CD (0.5 g each) were dried to constant mass at 65 °C and lead concentrations were determined by atomic absorption spectrometry (Hsu and Locke 1983) after digestion of the samples with concentrated nitric acid.

Isolation of the main lipid classes: The fresh leaves, CD or TM were homogenized with $CHCl_3$-MeOH (1:1) and refluxed for a few min in order to inactivate the lipases. Purification of the extracts was performed according to the modification of Bligh and Dyer (Christie 1973).

A part of the total lipid extract, containing 40 - 60 mg lipids, was separated by preparative TLC (silica gel G, *Merck*) with chloroform-methanol-acetone-acetic acid (35:7:12:0.2). The spots of the main lipid classes: TAG, MGDG, DGDG, SQDG and PL were located under UV-light after spraying with a fluorescent indicator (Popov and Stefanov 1968) and the lipids were determined quantitatively by gas chromatography with internal standard, as described earlier (Elenkov *et al.* 1993).

Results and discussion

Accumulation of lead in spinach leaves: The lead content in spinach leaves, CD and TM is proportional to the lead concentration in nutrient solution and to the period of incubation (Table 1). The treatment of intact leaves with lead acetate in concentration 3.8 mg dm^{-3} results in increase of the lead content in leaves. CD accumulate lead

Table 1. Accumulation of lead (mean ± S.E.; $n = 5$) in whole leaves, thylakoid membranes and cell debris of spinach after 56 and 64 d treatment with lead acetate.

Lead acetate [mg dm^{-3}]		Lead content [mg kg^{-1}(d.m.)] 56 d	64 d
0	whole leaves	1.40±0.04	1.01±0.06
	thylakoid membranes	0.17±0.01	0.12±0.01
	cell debris	0.89±0.06	0.98±0.03
3.8	whole leaves	1.86±0.08	2.51±0.12
	thylakoid membranes	0.79±0.02	1.18±0.04
	cell debris	0.89±0.06	1.13±0.08
38.0	whole leaves	10.90±0.22	11.32±0.44
	thylakoid membranes	3.11±0.06	3.36±0.06
	cell debris	7.20±0.04	7.66±0.12

slower than thylakoids, but the higher lead accumulation appeared at higher lead acetate concentration in nutrient solutions. Probably the protection of thylakoid membranes, essential for photosynthesis, is of great importance for the plants and apparently there are some protective mechanisms against accumulation of lead in them.

Effect of lead and sodium acetate on the acyl lipids: After the incubation of the plants with lead and sodium acetate there were no changes in the wet and dry matter of leaves and isolated membranes (data not shown). The lipid composition of the control plant is in accordance with the literature data (Quinn and Williams 1983, Webb and Green 1991). The main acyl lipid class is MGDG, followed with DGDG.

Thylakoid membranes: Surprisingly, there were not statistically significant differences between the total lipids of thylakoid membranes after incubation with lead and sodium acetate (Table 2), which is an indication that in this case the effect of metal ions is not specific.

Table 2. Effect of lead and sodium acetate on the acyl lipid composition (mean ± S.E.) in thylakoid membranes of spinach after 56 and 64 d treatment

	[mg dm^{-3}]	MGDG	DGDG	SQDG	PL	Total
56 d						
Control	0	7.3±0.7	4.8±0.5	3.0±0.3	2.6±0.2	17.7±1.7
Lead acetate	3.8	5.2±0.6	6.2±0.6	4.9±0.5	1.6±0.2	17.9±1.9
Lead acetate	38.0	4.8±0.5	6.4±0.6	5.5±0.5	1.4±0.2	18.1±1.8
Sodium acetate	2.8	5.0±0.6	6.6±0.6	5.0±0.5	1.0±0.1	17.6±1.8
Sodium acetate	28.0	4.6±0.5	6.2±0.5	5.4±0.5	1.2±0.1	17.4±1.6
64 d						
Control	0	7.4±0.7	4.7±0.4	3.2±0.3	2.6±0.2	17.9±1.6
Lead acetate	3.8	5.0±0.6	5.9±0.6	4.9±0.5	1.4±0.1	17.2±1.8
Lead acetate	38.0	4.5±0.4	6.3±0.7	6.2±0.6	1.2±0.1	18.2±1.8
Sodium acetate	2.8	4.8±0.4	5.8±0.5	6.4±0.6	1.0±0.1	18.0±1.6
Sodium acetate	28.0	4.6±0.4	6.2±0.6	6.0±0.5	1.4±0.2	18.2±1.7

Compared with the control there was no difference in the total lipid concentrations, but there were statistically significant differences in the amounts of the main lipid classes. The amount of MGDG was reduced and those of other glycolipids increased. Such a decrease of MGDG amount in thylakoid membranes was observed earlier after exposure of spinach to copper (Maksymiec *et al.* 1992) and of tomato plants to cadmium (Krupa and Baszynski 1989) ions. It is known (Kuiper 1984) that because of the hexagonal(2)-structure of MGDG, a reduced level of MGDG may indicate a higher degree of control on ionic permeability in the membranes and this may have an adaptive value.

The lead and sodium acetate treatment caused a substantial decrease in the amount of PL.

Cell debris: Analogously to thylakoid membranes, the lead and sodium acetate had equal effect on the lipid composition, but changes are different from those in thylakoid membranes (Table 3). In cell debris the treatment with lead and sodium acetate increased the MGDG and decreased the DGDG amounts. Analogously to thylakoid membranes, treatment with both acetates resulted in a decrease of PL concentrations, more significant when lead acetate was used.

Table 3. Effect of lead and sodium acetate on the acyl lipid composition (mean ± S.E.) in cell debris of spinach after 56 and 64 d treatment

	[mg dm^{-3}]	TAG	MGDG	DGDG	SQDG	PL	Total
56 d							
Control	0	9.4±0.8	6.2±0.7	4.1±0.4	2.6±0.2	6.3±0.5	28.6±2.6
Lead acetate	3.8	9.2±0.9	9.1±0.9	3.5±0.3	2.9±0.3	3.6±0.3	28.3±2.7
Lead acetate	38.0	9.8±0.8	9.7±0.9	3.1±0.3	2.7±0.2	2.4±0.3	27.7±2.5
Sodium acetate	2.8	10.2±1.0	8.8±0.8	3.2±0.2	2.2±0.2	5.7±0.4	30.1±2.6
Sodium acetate	28.0	8.7±0.8	9.2±0.9	2.8±0.2	2.9±0.2	4.3±0.4	27.9±2.5
64 d							
Control	0	10.8±1.0	6.4±0.6	4.5±0.4	2.8±0.2	9.3±0.9	33.8±3.1
Lead acetate	3.8	9.9±0.9	9.7±0.9	3.3±0.3	2.6±0.2	4.4±0.4	29.9±2.7
Lead acetate	38.0	10.2±1.0	9.9±0.9	3.1±0.3	2.2±0.2	6.3±0.5	31.7±2.9
Sodium acetate	2.8	11.1±1.0	10.0±0.9	2.8±0.2	2.8±0.2	8.7±0.8	35.4±3.2
Sodium acetate	28.0	10.7±1.0	9.7±0.9	2.6±0.2	2.6±0.2	8.3±0.8	33.9±3.1

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