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Lead uptake, localization and changes in cell ultrastructure of *Funaria hygrometrica* protonemata

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Abstract

The main sites of lead entering the protonemata were spores with broken cell walls and apical cells of protonemata. The lead ions could enter the symplast probably by endocytosis but we can not exclude diffusion as the way of entering. Lead deposits inside a protoplast were observed after 2 h of lead application. After 12 h, Pb deposits were commonly present in all cell structures surrounded by a membrane. Some disturbances in ultrastructure of organelles were found after 8 h. The most frequent were: change of endoplasmatic reticulum configuration from linear to concentric one in many cases surrounding other organelles (*e.g.* mitochondria) and large and numerous plastoglobuli in chloroplasts. Longer treatment of protonemata with lead (24 h or 48 h) caused degeneration of organelles and even death of the cell.

Additional key words: cell wall, chloroplast, endoplasmatic reticulum, Golgi apparatus, heavy metal, mitochondria, nucleus, nucleolus, Pb.

Introduction

Because of the permanent increase of industrialization and traffic the problem of lead contamination is still current especially in countries where the main source of lead is burnt gasoline supplemented with Pb. Generally Pb is not mobile element; more than 90 % of it is accumulated in root and only 10 % can reach the stem of plant and cells containing chloroplasts (*e.g.* Samardakiewicz and Woźny 1995).

Mosses may function as an environment indicators (Von Roloff 1989). They are very sensitive to different kinds of contaminations because they have not a protective wax coating. They take up water directly through their leaflets or thalli. Moreover moss protonema seems to be especially useful for the study of lead effects on chloroplasts. Protonemata laying on the surface of metal solution have a direct

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contact with the toxic element. Metal ions together with some essential substances are taken up by all cells of the thread. In this way they enter directly from solution to cells containing chloroplasts.

Therefore the aims of our studies were determination of the site of lead entering the cells, localization of this metal deposits in organelles, and possible disturbances in their ultrastructure.

Materials and methods

Protonema of moss *Funaria hygrometrica* Hedw. cultivated *in vitro*, on Kofler (1959) medium supplemented with microelements according to Heller (1953) was used. 5- or 6-d-old threads were treated with $PbCl_2$ (concentration of lead and incubation time were as following: 30 μM Pb: 48 h, 250 μM Pb: 6 - 24 h, 1000 μM Pb: 5 min - 4 h). We used two methods to detect lead deposits and to study its toxic effects in cells: rhodizonate method (Glater and Hernandez 1972) and transmission electron microscopy (TEM). For TEM study protonemata were fixed in the mixture (v:v) of 1.25 % glutaraldehyde and 1 % paraformaldehyde in 0.1 M cacodylic buffer, then postfixed in 1 % osmium tetroxide in the same buffer, and embedded according to Spurr (1969). Ultrathin sections were observed with a *TEM - JEM 1200 Ex* (*JEOLCo*, Tokyo, Japan). TEM studies were concerned apical cells.

Results

Lead uptake and localization of its deposits in cell: We determined two sites of the protonema where lead deposits were detectable relatively early: spores with broken intine and exine and apical cell of protonemata. First deposits inside protoplast were found after 2 h (1000 μM Pb). After shorter time of incubation only lead deposits adsorbed to the surface of the cell wall were detectable. Characteristic invagination of plasma membrane containing lead deposits and the fact that in protoplast they were present only surrounded by membrane suggested us that lead should enter the protoplast by endocytosis.

These methods did not exclude the possibility of lead ions entering by diffusion and their presence in protoplast in dissolved form as well as determine the percentage of lead taken up by endocytosis and by diffusion.

First deposits of lead in cell were found after 2 h. Then they were present mainly in cell wall (Fig. 4), nucleus and nucleolus (Fig. 4), vesicles of different origin (Golgi apparatus, GA, vesicles - Fig. 2) and in stacked and unstacked thylakoids (Fig. 3), especially in these laying near plastid envelope. Generally the number of deposits was low. Apart from lead deposits presence no changes of cell ultrastructure were found in comparison with the control (Fig. 1). Longer treatment (3 and 4 h) caused little increase of deposits number in the same structures like after 2 h and additionaly their appearance in vacuole. After 8 h deposits in chloroplast were placed in similar number both in the outer and inner part of thylakoid system,

12 h treatment caused high increase of deposits number in organelles described above and moreover they were present in mitochondria envelope and cristae (inside the lumen and on the surface of membrane) (Fig. 8), nuclear envelope (Fig. 7), lumen of endoplasmatic reticulum (ER) (Fig. 10) and in cisternae of GA (Fig. 9). We did not found any deposits directly placed in ground cytoplasm. Incubation 24 and 48 h did not caused any differences in localization of lead deposits in cell.

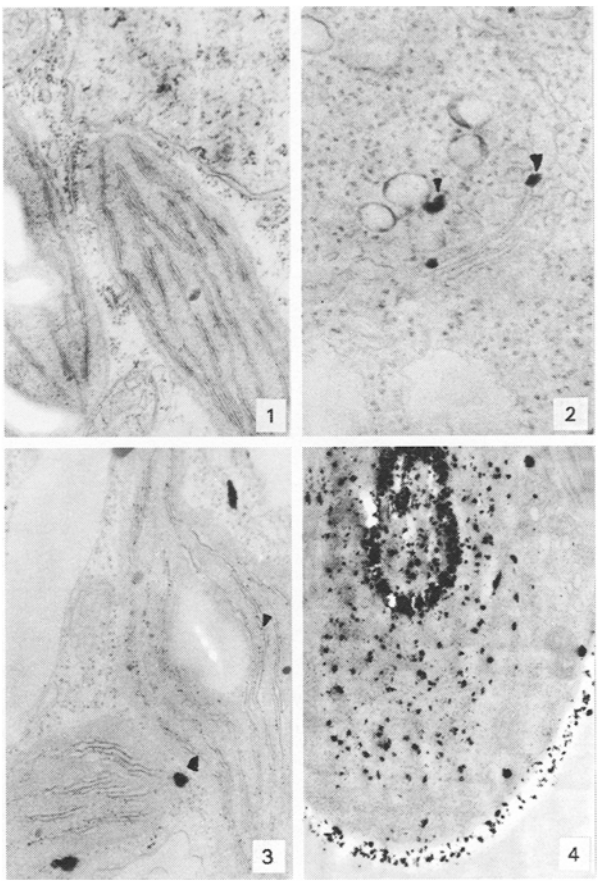

Fig. 1. Part of apical cell - control (incubation in water) (24 000 ×).

Figs. 2 - 4. Localization of lead deposits in apical cell of protonemata (treatment 2 h, concentration 1000 μM Pb): lead deposits in vesicles of Golgi apparatus (*arrowheads*), (Fig. 2; 75 000 ×), in thylakoid system (*arrowheads*) (Fig. 3; 30 000 ×), in cell wall, nucleus and nucleolus (Fig. 4; 18 000 ×).

Alteration of cell ultrastructure: During first 4 h of treating protonemata with lead in spite of lead deposits present in some organells no alterations of cell ultrastructure

were found. First ones appeared after 8 h. Then we found concentric forms of ER sometimes surrounding other organelles (*e.g.* mitochondria - Fig. 6), and relatively large and numerous plastoglobuli in chloroplasts (Fig. 5). After 12 h concentric forms were the dominant form of ER, moreover there were found some disturbances of GA structure, *e.g.*, cup shaped (Fig. 9). After 48 h Pb caused degradation of chloroplasts (Fig. 12) and swelled envelope of mitochondria (Fig. 11). Besides plastids which showed many disturbances in ultrastructure some cells contained

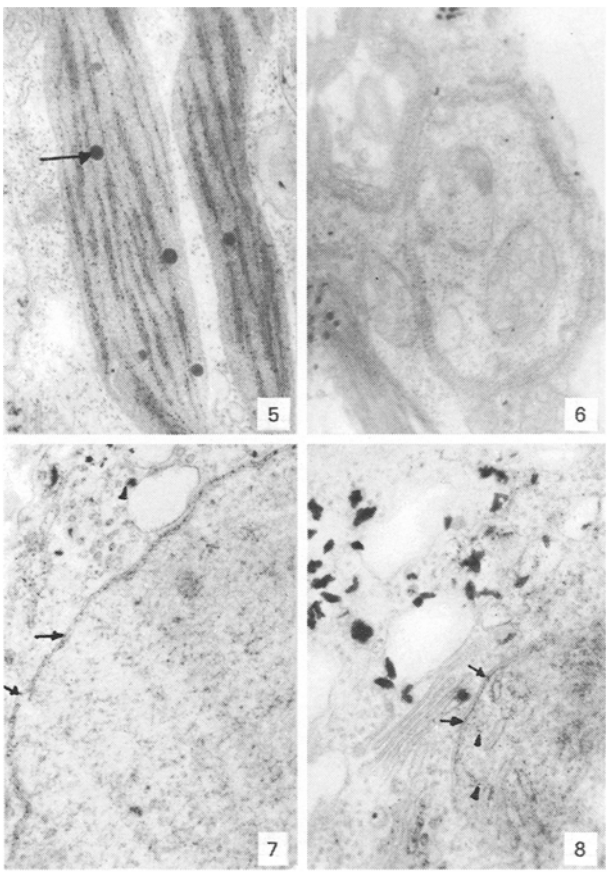

Fig. 5. Lead deposits in thylakoids system (treatment 8 h, concentration 250 μM Pb); relatively large and numerous plastoglobuli (*arrow*) (30 000 ×).

Fig. 6. Concentric forms of ER surrounding mitochondria (treatment 8 h, concentration 250 μM Pb) (28 000 ×).

Figs. 7 - 8. Lead deposits (treatment 12 h, concentration 250 μM Pb) in nucleus envelope (*arrows*), ER and vesicles (*arrowhead*) (Fig. 7; 45 000×), in mitochondrion envelope (*arrows*), cristae (*arrowheads*), cisternae of GA and different size of vesicles (Fig. 8; 60 000 ×).

only little changed plastids even after long time of treating protonemata with Pb. Relatively stable organelle in comparison with chloroplasts and especially mitochondria was nucleus. Apart from lead presence inside no more structural changes were observed even after 24 and 48 h of incubation.

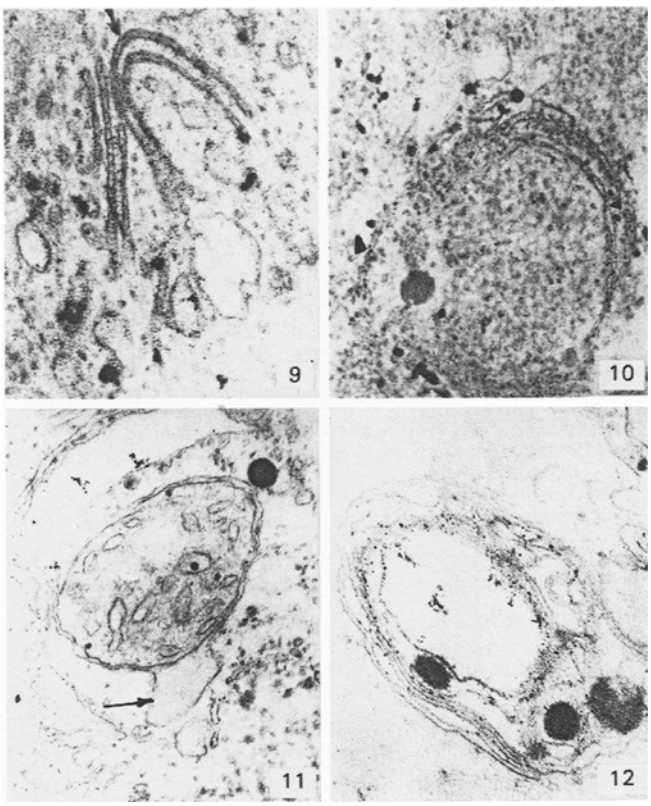

Fig. 9. Lead deposits (treatment 12 h, concentration 250 μM Pb) in cisternae and vesicles of GA; cup shaped of GA (*arrow*) (81 000 ×).

Fig. 10. Lead deposits (treatment 12 h, concentration 250 μM Pb) in lumen (*arrowheads*) of concentric form of ER (54 000 ×).

Fig. 11. Swelled envelope of mitochondrion (*arrow*) (treatment 48 h, concentration 30 μM Pb) (54 000 ×).

Fig. 12. Degenerated chloroplast (treatment 48 h, concentration 30 μM Pb) (57 000 ×).

Discussion

Lead ions can enter the cells of moss protonema at least in two ways by endocytosis and by diffusion. The facts that lead deposits were present mainly in organelles, *e.g.*

GA, ER, vacuole, suggest that lead ions enter the cell mostly by endocytosis.

Change of the linear configuration of ER to concentric one (often found in protonemata cells treated with Pb) is probably connected with preventing cell from lead toxicity. It is possible that concentric configuration makes the structure more effective in metal detoxification because in this form the relation of the area to the volume is better. Lead deposits in such configurations of ER were found not only in *Funaria* cells, but also in many other plants treated with this metal (Woźny 1987, Woźny *et al.* 1994). It seems to be possible regarding the appearance of ER in concentric form as a sympthom of adaptation rather than degeneration process.

Deposition of lead in vacuole, and cell wall after longer treatment (12 h - 48 h) might be compartmentation of it in metabolically less active structures, preventing in this way more sensitive sites in cytoplasm from metal toxicity.

Similarly to senescence (Butler and Simon 1968, Hillman *et al.* 1994) we also observed, but earlier, some alterations of size, shape and ultrastructure of plastids as the result of presence of toxic elements in the cell. Simultanously chloroplasts are rather stable because even in degenerated cell some of them sometimes stay a little disturbed. The presence of Pb deposits in thylakoids suggests that disturbances in ultrastructure of chloroplasts should be the effect of this metal toxicity. Similar changes were observed in other plant cells. More circular shape of plastid was observed, *e.g.*, in detached, ageing leaves of wheat (Shaw and Manocha 1965), or in cells of *Lemna minor* treated with Pb (Kaźmierczak 1990). Large sudanophilic and osmiophilic plastoglobuli were also found by Butler and Simon (1968), Młodzianiowski and Ponitka (1973).

Hence some degeneration disturbances of chloroplasts ultrastructure in moss protonemata are similar to the natural process of plastid senescence, however, they appear erlier.

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