Localization of Lead Accumulated by Corn Plants

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ABSTRACT

Light and electron microscopic studies of corn plants (Zea mays L.) exposed to Pb in hydroponic solution showed that the roots generally accumulated a surface Pb precipitate and slowly accumulated Pb crystals in the cell walls. The root surface precipitate formed without the apparent influence of any cell organelles. In contrast, Pb taken up by roots was concentrated in dictyosome vesicles. Dictyosome vesicles containing cell wall material fused with one another to encase the Pb deposit. This encaespated deposit which was surrounded by a membrane migrated toward the outside of the cell where the membrane surrounding the deposit fused with the plasmalemma. The material surrounding the deposit then fused with the cell wall. The result of this process was a concentration of Pb deposits in the cell wall outside the plasmalemma. Similar deposits were observed in stems and leaves suggesting that Pb was transported and deposited in a similar manner.

Many researchers have shown that plants will accumulate Pb either from soil, stem, or foliar application (1, 3, 8, 9, 13, 14, 16, 25). However, the reports conflict as to the amounts accumulated and the amounts that can be translocated. Litle has been done to localize the Pb accumulated within an organ, although Hammett (5–7) indicated that much of the Pb was associated with cell walls and nuclei, and Suchodoller (24) found that it was concentrated in the root cortex.

It was the purpose of this research to study the localization of accumulated Pb and characterize the method of accumulation as specifically as possible.

MATERIALS AND METHODS

Corn (Zea mays L. Wf9 × M14) was grown by a standard hydroponic procedure (19) in a greenhouse or was grown on paper toweling in a moist chamber. Pb was supplied in complete Hoagland's solution, or in distilled water either as a citrate or an EDTA chelate, or as PbCl₂ or Pb(NO₃)₂. The lead concentration ranged from 10 to 1000 mg/l. With the one exception noted later, the form of Pb and the method of exposure to Pb made no difference in the nature or sequence of events and will not be referred to again.

Specimens were fixed either in 2% glutaraldehyde in 0.1 M phosphate buffer, in Millonig phosphate buffer, in veronal acetate buffer, or in 0.2 M cacodylate buffer at pH 7.4, in 10% acrolein in distilled water, or in 1% KMnO₄, in water. Glutaraldehyde- or acrolein-fixed material was split into two groups, one of which received postfixation in 1% OsO₄ in the respective buffers, and the other received no postfixation. Dehydration was in a graded series of ethyl alcohols, and propylene oxide was used as a solvent for the Epon embedment (11). Specimens were either stained in uranyl acetate (23) and lead citrate (18) or were left unstained for observation on either a Siemens Elmiskop I, an RCA EMU-3F, or an RCA EMU-4 electron microscope.

RESULTS

Light Microscopy. Pb crystals were easily visible with phase contrast light microscopy. Numerous crystals were visible on the surface of the root (Fig. 1, arrow), with the exception noted below. On the interior of the root, crystals were numerous in the stele and seemed to be associated with cell walls (Fig. 2, arrows). Root tips also contained crystals which were slightly smaller than the crystals found in the stele in older portions of roots (Fig. 3, arrows). Similar crystals could also be found in leaves (Fig. 4, arrows). Throughout the plant the crystals were so small that a thorough study necessitated the use of the electron microscope.

Electron Microscopy. Within 1 hr after the introduction of Pb to the hydroponic solution, there was a precipitate of Pb on the root's surface. The amount of Pb precipitate increased with time for approximately 6 hr (Fig. 5). At this time most of the Pb that would precipitate on the surface was present. The only exception occurred when EDTA was used to chelate the Pb, and no precipitate was ever observed on the root's surface regardless of the amount of Pb in solution (Fig. 6). This is in contrast to the case in which Pb was chelated with citrate (Fig. 7).

When present, the surface precipitate consisted of large angular crystals 1 to 3 μm long (Fig. 5). These crystals occurred as far as 10 μm from the root surface. Just along the surface of the cell wall there were small, spherical, amorphous deposits 100 to 250 nm in diameter. Within the wall itself, small irregular deposits measuring 3 to 10 nm were apparent. These deposits were observed regardless of the fixative or buffer system used.

Examination of root tips revealed that within 2 hr after the introduction of Pb, regardless of the chelating agent, dense deposits appeared in dictyosome vesicles throughout the root (Fig. 8). Not all of a dictyosome's vesicles contained the deposits. Deposits were more frequent toward the secreting face than the forming face, although some were observed on the forming face. Hypertrophied dictyosomes of the root cap contained fewer deposits than the more normal dictyosomes toward the center of the root. However, some secretion products of the hypertrophied vesicles that had passed through the plasmalemma did contain many dense deposits. Vesicles containing Pb deposits were never observed fusing with any other.

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FIGS. 1-4. Phase contrast light micrographs showing lead crystals (arrows). Line scales represent 10 μm in all Figs. Fig. 1. Surface deposits on root treated with 1000 mg/l Pb as Pb citrate. Fig. 2. Deposits in stele of root treated with 250 mg/l Pb as Pb citrate. Fig. 3. Root tip treated with 250 mg/l Pb as Pb citrate. Fig. 4. Leaf with deposits in cell walls.
Figs. 5-9. Transmission electron micrographs of corn roots. Line scales represent 1 μm in all Figs. Fig. 5. Cross section of root showing three types of deposits (arrows). Fig. 6. Cross section of root tip treated with 250 mg/l Pb as Pb EDTA. Note lack of deposits. Fig. 7. Cross section of root tip treated with 250 mg/l Pb as Pb citrate. Note deposits. Fig. 8. Root tip treated with PbCl₂ for 2 hrs. Note dense deposits in dictyosome vesicles (arrows). Fig. 9. Small deposit in root after 6 days in 250 mg/l Pb as Pb citrate.
Dense deposits were never observed in mitochondria, plastids, or nuclei. Occasionally, dense deposits were observed in the proximity of boundary formations and between membranes of endoplasmic reticulum. However, the deposits associated with these two cytoplasmic components were much less frequent than those associated with dictyosomes.

After longer exposure to Pb, the deposits became larger (Fig. 9). These larger deposits consisted of a group of crystals encased in what appeared to be cell wall material and surrounded by a unit membrane (Fig. 10, arrow). Dictyosome vesicles containing cell wall precursors were observed fusing with the deposits (Fig. 11, arrows). The usual size of the deposits was 1 to 2 μm wide by 3 to 5 μm long.

Larger deposits were observed at the periphery of cells (Fig. 12) where the membrane surrounding the deposit fused with the plasmalemma (Fig. 13, arrow). Subsequently, the cell wall material surrounding the deposit fused with the cell wall (Fig. 14, arrow). The cell wall at the site where crystals had been deposited was abnormally thick due to the extra cell wall material that had been deposited with the crystals (Fig. 15). Often the cell wall and the crystal protruded some distance into the cell. These crystalline deposits were clearly visible with the light microscope and often tended to be opposite one another in adjacent cells (Figs. 3 and 5).

Crystals with similar morphology and associated with uneven, protruding cell walls were observed throughout the root system (Figs. 1–4, arrows, and Fig. 16), in stems, and in leaves of corn plants (Fig. 17). In the roots the crystals were most prevalent in, but not limited to, the stele, while in the stems and leaves they tended to be in or close to vascular bundles. More crystals were present in roots than in stems, and stems contained more crystals than did leaves. Crystals were observed to be associated with every cell type except phloem sieve tube and companion cells, and epidermal guard and accessory cells. Crystals were observed with treatment levels as low as 10 mg/1 Pb. While the frequency of deposit occurrence increased with increasing Pb levels, crystal morphology, method of deposition, and final location in the cell remained constant.

Table I presents the amount of Pb accumulated by roots and the number of crystals present in 1 mm of root tip. Note that as Pb content increased, the number of crystals also increased.

**DISCUSSION**

There are many techniques that utilize lead- and phosphorus-containing compounds to localize phosphatases, or use Pb alone to localize inorganic phosphate (4, 10, 20–22). The pattern of Pb deposition here is different than anything reported previously where Pb was deposited during or after fixation. Walton (26) has demonstrated that isolated animal mitochondria will accumulate Pb granules. Unpublished ultrastructural research in this laboratory has found that corn shoot mitochondria will also accumulate lead in vitro. However, we have not been able to demonstrate lead accumulation in vivo by corn mitochondria. There appears to be an organelle, the dictyosome, which has a much higher affinity for lead than does the mitochondrion.

The precipitates on the surface of the root contrast in both size and mode of formation to the larger deposits in the interior of the root. These surface precipitates apparently form rapidly and without the mediation of any cell organelle. Since the larger one is crystalline and the smaller one is amorphous, it would seem reasonable to assume that at least two different compounds are involved. Whether either one of these represents a root exudate reacting with Pb is unknown.

It has been demonstrated that only some of the dictyosome vesicles yield the initial Pb precipitates. The reason for Pb precipitating in some vesicles and not in others is not understood at this time. Cytologists have long suspected that not all dictyosome vesicles are alike (2). In corn specifically, some vesicles appeared to be empty while others appeared to be filled with normal secretion products. Practically no Pb precipitated in the hypertrophied vesicles of the root cap, but secretion products that accumulated between the plasmalemma and the cell wall in root cap cells did sometimes contain precipitates. Most of the vesicles in the remainder of the root that contained Pb deposits also appeared to contain normal secretion products. This suggests that it is not the cell wall precursors alone that bind the Pb. Possibly only those cytoplasmic components which contain sufficient concentrations of certain anions, those which form insoluble precipitates with Pb, could act as sinks for Pb. For example, dictyosomes would have a high phosphate content since they contain acid phosphatase.

Once Pb has initially precipitated in a vesicle, regardless of whatever conditions preceded precipitation, the following sequence of events occurred. Cell wall precursors were added to the vesicle that contained the Pb crystals. This was done either by apposition of vesicles containing cell wall secretion products (Fig. 8) or by the vesicle containing the Pb itself starting to secrete cell wall compounds. The crystal grew, and as it did, more cell wall materials were added. Eventually these deposits moved to the periphery of the cell where they passed through the plasmalemma and fused with the cell wall. It is not clear whether or not a crystal can continue to grow after being deposited in a cell wall.

While the sequence of events concerning the deposits of crystals was obtained from root tips, there was evidence to suggest that this occurred throughout the plant. Crystals with similar morphology were present in stems and leaves. Wherever these crystals were deposited, the cell walls were always unevenly thickened and always protruded into the cell. Dictyosomes were present throughout the plant, and it is reasonable to assume that dictyosomes of stems and leaves had the same capabilities as those of roots. Furthermore, for crystals to be formed in the first place, Pb had to be mobile enough to move through the cytoplasm to reach the dictyosome. If Pb was mobile in the root, then it is certainly possible that some of that mobile Pb, possibly only a small fraction of the total, could move throughout the rest of the plant and be precipitated.

Thus, it is possible that there were at least two forms of Pb present: an insoluble or immobile form (accounting for most of the Pb), and a soluble or mobile form (a minor fraction of the total Pb). Since dictyosome precipitates form soon after the introduction of Pb, the latter form appears to have moved relatively freely through the cytoplasm until it reached a sink, in this case a dictyosome vesicle.

This ability to concentrate Pb is in strong contrast to the role normally assigned to dictyosomes: that of compound secretion and cell wall deposition (2, 15, 17). This is the first experiment that we are aware of in which the dictyosome appears to participate in the crystallization and removal of a toxic compound from the cell cytoplasm. Crystallization of toxic compounds normally occurs in vacuoles of special cells and in association with elaborate membrane systems (2). There are two possible interpretations: (a) Dictyosomes normally function in the sequestering of unwanted compounds from the cell, and it was only readily apparent here due to the electron dense nature of the Pb; or (b) dictyosomes precipitated the Pb only incidentally as a result of their normal activities, and any cations that would form sufficiently insoluble precipitates would react the same way.

It is not possible to say which of these two interpretations is best at this time. However, our understanding of the dictyosome
Figs. 10–13. Transmission electron micrographs of lead deposits in corn roots after 6 days of treatment. Line scales in all Figs. represent 1 μm. Fig. 10. Small deposit surrounded by a membrane (arrow). Fig. 11. Dictyosome vesicles containing cell wall material fusing with a deposit (arrows). Fig. 12. Deposit nearing cell wall. Fig. 13. Membrane surrounding deposit fusing with plasmalemma (arrow).
Figs. 14–17. Transmission electron micrographs of lead deposits in corn plants. Line scales represent 1 μm in all Figs. Fig. 14. Wall around deposit fusing with wall of root cell (arrow). Fig. 15. Typical appearance of deposits in root tip cell walls. Note unevenly thickened cell wall. Fig. 16. Typical appearance of deposits in older portions of root. Fig. 17. Deposits in corn leaf. Note relationship to cell wall.
Table I. Lead Content and the Number of Crystal Deposits in Hydroponically Grown Roots

<table>
<thead>
<tr>
<th>Lead as Citrate in Solution</th>
<th>Oven Dry Weight</th>
<th>Crystals in 1 mm of Root Tip</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/l</td>
<td>µg of lead/g</td>
<td>approximate no.</td>
</tr>
<tr>
<td>10</td>
<td>3,300</td>
<td>250</td>
</tr>
<tr>
<td>50</td>
<td>5,600</td>
<td>2,500</td>
</tr>
<tr>
<td>250</td>
<td>30,000</td>
<td>12,500</td>
</tr>
</tbody>
</table>

1 Determined for whole roots by atomic absorption after two 15-min washes in 20 mm EDTA to remove surface lead.

2 Calculated by surveying 1 µm thick plastic sections with phase contrast optics.

is probably oversimplified. Manton and Ettl (12) have studied the dictyosomes of some algae and found that each of three types of scale found on the alga was synthesized in a separate dictyosome vesicle. Some of these scales were very ornate, three-dimensional structures that would require a great deal of precision to assemble. It is therefore possible that we have not yet come to understand the versatility and complexity of the dictyosome.

This study has shown that if Pb is available to a corn plant, the root system will take up Pb and the Pb will be transported and precipitated throughout the plant. In sequestering Pb from its own cytoplasm, the plant has concentrated large quantities of Pb in a relatively small area. There are two important points to be made. First, simply because the Pb was sequestered it cannot be assumed that the Pb did not adversely affect the physiology, productivity, and efficiency of the plant. Second, if Pb became available to corn under natural conditions, the plant's ability to concentrate Pb could have serious effects on organisms further down the food chain. Not only do people and economically important animals use parts of corn for food, but a whole range of insects and small animals also use corn as a food source.

LITERATURE CITED