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Subcellular Localization and Characterization of Excessive Iron in the Nicotianamine-less Tomato Mutant *chloronerva*[1]

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To understand the function of the Fe^{2+}-complexing compound nicotianamine (NA) in the iron metabolism of plants we have localized iron and other elements in the NA-containing tomato wild type (*Lycopersicon esculentum*) and its NA-free mutant *chloronerva* by quantitative x-ray microanalysis. Comparison of element composition of the rhizodermal cell walls indicated that the wild type accumulated considerable amounts of iron and phosphorus in the cell wall, whereas in the mutant iron and phosphorus were detected in the cytoplasm and vacuoles of the rhizodermis. In mutant leaves containing high iron concentrations in the symplast, electron-dense inclusions were detected in chloroplasts and phloem. Such particles, consisting mainly of iron and phosphorus, were never found in the wild type and were very rarely detected in young chlorotic mutant leaves or after treatment of the mutant with NA. For further characterization the electron-dense inclusions in mutant leaves were isolated and compared by sodium dodecyl sulfate-gel electrophoresis and immunoblotting to ferritin from iron-loaded *Phaseolus vulgaris* leaves. Antibodies raised against purified *Phaseolus* leaf ferritin were used. Neither in mutant nor in wild type (iron loaded and control) was ferritin protein detected. These results suggest that the electron-dense inclusions in mutant leaves are not identical with ferritin. It is concluded that NA is necessary to complex ferrous iron in a soluble and available form within the cells. In the absence of NA the precipitation of excessive iron in the form of insoluble ferric phosphate compounds could protect the cells from iron overload.

NA, a nonprotein amino acid, is a key substance in the biosynthesis of mugineic acid-family phytosiderophores (Shojima et al., 1990). In contrast to phytosiderophores that complex Fe^{3+} (Takagi, 1976; Kawai et al., 1988), NA forms stable complexes with Fe^{2+} and other divalent transition metal ions (Beneš et al., 1983; Anderegg and Ripperger, 1989). This complex formation with divalent transition metal ions seems to be the primary function of NA within plants (Schreiber, 1986; Scholz et al., 1988). NA is universally distributed within the plant kingdom. The tomato (*Lycopersicon esculentum*) mutant *chloronerva* is the only known NA-free higher plant (Rudolph et al., 1985). Retarded growth of shoots and roots and interveinal chlorosis of the youngest leaves are typical characteristics of the mutant, which can be supplemented by application of NA either to the nutrient solution or to the leaves (for reviews, see Scholz et al., 1988, 1992). The mutant translocates large amounts of iron to the shoot, leading to an accumulation of iron, especially in older leaves (Scholz et al., 1985; Becker et al., 1992). Despite this iron accumulation, the mutant exhibits typical iron deficiency-induced responses, for instance extrusion of protons by roots, thickened root tips, increased ferric reductase activity in roots (Stephan and Grün, 1989), and citrate accumulation in different parts of the plant (Pich et al., 1991). This apparent iron deficiency of the mutant is not due to an inhibited transport of iron through the plasmalemma into the cell. Investigations of apoplasmic and symplasmic iron concentrations demonstrated that roots and leaves of the mutant contain significantly higher symplasmic iron concentrations compared to the wild type (Pich and Scholz, 1991; Becker et al., 1992).

The aim of the present study is to answer the questions of where, and in which form, the excess in iron is located in the mutant and why it is not sufficiently available for the *chloronerva* mutant. In this investigation we used x-ray microanalysis for the subcellular localization of iron and other elements. Additionally, electrophoretic and immunological investigations were used to characterize the localized iron.

MATERIALS AND METHODS

Plant Material and Culture

Tomato seedlings (*Lycopersicon esculentum* Mill. cv Bonner Beste and its mutant *chloronerva*) were grown in a controlled-environment chamber with a 16-h, 25°C/8-h, 18°C day/night regime; a RH of 70 to 75%; and irradiance (at 400–700 nm) of 225 μE m^{-2} s^{-1}. Seedlings were cultivated hydroponically in an aerated nutrient solution containing the following macro- and micronutrients: 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 1 mM KH_2PO_4, 1 mM $MgSO_4$, 50 μM H_3BO_3, 4.5 μM $MnCl_2$, 3.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, and 0.1 μM $(NH_4)_6Mo_7O_{24}$. If not otherwise stated, iron was supplied as FeEDTA (10 μM). The pH of all nutrient solutions was adjusted with KOH to 5.5. Nutrient solutions were changed every 2 d.

For the isolation of ferritin, leaves of iron-loaded beans (*Phaseolus vulgaris* L.) were used (see below). Additional

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Abbreviations: EDAX, energy-dispersive x-ray microanalysis; NA, nicotianamine; TEM, transmission electron microscope.

plant species cowpea (*Vigna unguiculata*), barley (*Hordeum vulgaris*), and poinsettia (*Euphorbia pulcherrima*) were used to test the cross-reactivity of the ferritin antiserum. These species were grown under greenhouse conditions.

Culture of Iron-Loaded Plants

To obtain leaves with high iron content, a short-term overdose of iron was supplied to iron-deficient plants (Seckbach, 1969). Seeds of *P. vulgaris* L. were allowed to imbibe overnight in tap water and then germinated for 7 d in the dark on polyvinyl chloride needles, moistened with distilled water. Subsequently, seedlings were transferred for 8 to 9 d to 1-L vessels containing aerated iron-free nutrient solution (for nutrient solution and growth conditions, see above). Afterward, the chlorotic plants were transferred for 3 to 4 d to nutrient solution containing 90 μM FeEDTA. Control plants were cultivated with 40 μM FeEDTA. The first trifoliate leaves from 20- to 21-d-old plants were used for ferritin isolation.

Tomato seedlings were grown for 2 d in nutrient solution containing 10 μM FeEDTA before they were transferred to iron-free nutrient solution for 6 to 7 d. The chlorotic plants were then transferred for 3 to 4 d to nutrient solution containing 50 and 90 μM FeEDTA, respectively. Control plants were cultured with 10 μM FeEDTA.

NA Application

Beginning at the time of primary leaf emergence (9th d), 0.5 mM NA solution was applied three times per day to the leaves of mutant plants. During the application period of 11 d, each plant received approximately 1 μmol of NA in total. NA prepared from mature seeds of *P. vulgaris* L. was provided by Dr. K. Seifert (Institut für Pflanzenbiochemie, Halle, Germany).

EDAX

Roots and leaves from 20-d-old plants of *L. esculentum* (wild type, mutant, and mutant + NA) and *P. vulgaris* (control and Fe-loaded) were cut to 1- to 2-mm pieces, rapidly frozen in a 2:1 mixture of propane:isopentane at −196°C (Jehl et al., 1981), freeze-dried at −50°C, and stored over silica gel. Root tissue was taken from approximately 1-cm-long root tips and distal zones of lateral root formation. The root and leaf pieces were infiltrated with diethyl ether under vacuum and pressure and embedded in styrene-methacrylate as described by Fritz (1989). Sections 1 μm thick were cut with an ultramicrotome using dry glass knives (Ultracut E, Reichert-Jung, Vienna, Austria), mounted on adhesive-coated 100-mesh hexagonal grids (Fritz, 1991), coated with carbon, and stored over silica gel until analysis.

The sections were analyzed in a Philips (Eindhoven, The Netherlands) EM 420 TEM with the energy-dispersive system EDAX 9100. Spectra were usually collected at a magnification of 6350×. The accelerating voltage was 120 kV, the take-off angle was 25°, and the counting time was 60 live seconds.

For analyzing cell compartments, the electron beam was adjusted to a size not larger than the structure of interest. For example, most cell walls were analyzed with an electron beam 250 nm in diameter. For analyzing chloroplasts or vacuoles, the electron beam was widened to cover a large number of eutectic structures of the compartment. At least two sections from two different tissue blocks of each treatment or plant were analyzed; for each tissue compartment, five to seven measurements per section were carried out. The data of the two sections were pooled and the means and SD values were determined.

The x-ray spectra were processed with the SQ program of the EDAX-9100 software after manual adjustment for background. Quantitative data were obtained by comparing the peak integrals of the elements with the K peaks of agar standards containing known K amounts (Fritz and Jentschke, 1994), taking into account the calibration coefficients (Cliff-Lorimer factors) of the elements relative to K.

Isolation and Purification of Ferritin

Ferritin was isolated from iron-loaded and normal leaves of bean, tomato (wild type and mutant), barley, cowpea, and poinsettia by modification of the procedures of van der Mark and van den Briel (1985). Freshly harvested leaves (20 to 40 g) were ground in liquid nitrogen with a mortar and pestle. The powder was transferred into 5 volumes of ice-cold extraction buffer (50 mM Tris-HCl, pH 7.3, 1 mM DTT, 2% [w/v] PVP) and the mixture was homogenized for 1 min with an Ultra-Turrax (Janke and Kunkel, Staufen, Germany) at maximum speed. The slurry was filtered through eight layers of mull plus one layer of nylon gauze and centrifuged at 9,000*g* for 15 min. The supernatant was adjusted to 50 mM $MgCl_2$, centrifuged for 10 min at 2,000*g*, and then brought to 50% saturation with solid ammonium sulfate. The resulting protein pellet was dissolved in buffer A (50 mM Tris-HCl, pH 7.3, 0.1 mM DTT, 0.05% [w/v] trichlorobutanol), dialyzed against the same buffer, and applied to a DEAE-Sepharose (Pharmacia) column (2 × 25 cm) equilibrated with buffer A. The column was washed until no *A* was detected in the eluate at 280 nm. The ferritin was eluted with a linear gradient of 0 to 0.5 M NaCl in buffer A. The iron-containing fractions were pooled and centrifuged at 100,000*g* for 16 h at 4°C in a 60 Ti rotor (Beckman). The supernatant was removed and ferritin-containing pellets were resuspended in buffer A and, when not used directly for further purification, adjusted to 60% (v/v) glycerol and stored at −20°C. Ferritin iron concentration was estimated by measuring *A* of the Fe(II)[bathophenanthroline disulfonic acid, Na_2-salt]$_3$ complex at 540 nm, using sodium dithionite as a reducing agent.

To get sufficient amounts of ferritin for the preparation of antibodies, the modified method of Lane and Skopp (1986) was used. Twenty to 40 g of leaves were ground in liquid nitrogen. The resulting powder was then added to 6 volumes of 0.1 M diammonium citrate buffer, pH 6.5, containing 0.04% (w/v) iodoacetamide and 5 mM sodium diethyldithiocarbamate and was homogenized for 1 min in an Ultra-Turrax at maximum speed. The homogenate was filtered through eight layers of mull plus one layer of nylon

gauze and centrifuged at 9,000*g* for 15 min. The supernatant was mixed with a 10% (w/v) SDS solution in a 5:1 ratio. Twenty milliliters of this solution was introduced into a polycarbonate centrifuge tube and underlayed with 7 mL of 20% (w/v) Suc in the ammonium citrate buffer (no additives). The samples were centrifuged for 3 h at 100,000*g* at 20°C. The supernatant was discarded, and the brown pellets were resuspended in small amounts of 0.05 M Tris-HCl buffer, pH 7.4, and used for gel electrophoresis or the preparation of antisera.

Gel Electrophoresis

SDS-PAGE (12%) was performed on 1-mm-thick slabs according to Laemmli (1970). Three to 10 μg protein was applied per lane. Electrophoresis was carried out for about 1 h at 100 V until the tracking dye (bromphenol blue) reached the bottom of the gel. The gel was then fixed in 20% (v/v) methanol, 7% (v/v) acetic acid for 1 h and stained in 0.06% (w/v) Coomassie blue R-250 in the same solvent for 1 h. The gel was destained in 30% (v/v) methanol, 5% (v/v) acetic acid.

The mol wt of native protein and the purity of ferritin preparations were assessed by 5% PAGE in a nondenaturing system.

Preparation of Antiserum and Protein Immunoblotting

Antiserum was prepared in mice that were primed and boosted three times over a space of 6 weeks by intraperitoneal injections with purified ferritin preparations from Fe-loaded *P. vulgaris* leaves. Antibodies were raised from ascites fluid of immunized mice and the immunoglobulin fraction was isolated by ammonium sulfate precipitation (33% saturation) followed by affinity chromatography on goat anti-mouse IgG covalently linked to glutaraldehyde-activated affinity adsorbents (Boehringer Mannheim). For control experiments, mouse IgG was isolated from preimmune mouse sera by the same procedure.

For western blot analysis, enriched ferritin preparations from different plants were separated on 12% polyacrylamide gels under denaturing conditions (Laemmli, 1970) and electrophoretically transferred to nitrocellulose membranes (Schleicher and Schüll BA 85, 0.45 μm pore size) according to Towbin et al. (1979). Filters were blocked for 4 h in 3% (w/v) BSA and incubated for 1 h with a 1:1000 dilution of primary antibody in 10 mM Tris-HCl, pH 7.4, supplemented with 0.9% (w/v) NaCl, 1% (w/v) BSA, and 0.5% (v/v) Tween 20. After they were washed in 10 mM Tris-HCl containing 0.9% (w/v) NaCl and 1% (v/v) Triton X-100, the membranes were incubated for 1 h in a 1:3000 dilution of sheep anti-mouse horseradish peroxidase-conjugated IgG (Sigma) and washed again. Immunodetection was performed with an ECL western blotting detection reagent according to the manufacturer's protocol (Amersham).

RESULTS

Leaf and root preparations of wild-type tomato, NA-free mutant, and NA-treated mutant were analyzed by means of x-ray microanalysis with a TEM. In the older, completely green mutant leaves, which contained high symplasmic iron concentrations (Becker et al., 1992), electron-dense inclusions could be detected in chloroplasts of palisade parenchyma and in phloem. Typical x-ray spectra from such electron-dense particles are shown in Figure 1. These particles consist mainly of iron and phosphorus (Fig. 1, A and C). Iron was not detectable outside of these particles (Fig. 1, B and D). We never found comparable electron-dense deposits in wild-type leaves, and we found them very rarely in young chlorotic mutant leaves or after treatment of the mutant with NA.

Tomato roots have the capacity to accumulate substantial amounts of iron and phosphorus in their cell walls (Fig. 2). In the rhizodermal cell walls of the NA-free mutant, such iron and phosphorus accumulations were absent. Instead, the mutant accumulated iron and phosphorus in the cytoplasm and vacuoles of the rhizodermis. Moreover, increased amounts of manganese and zinc were observed in vacuoles. After treatment of the mutant with NA, the element composition of rhizodermal cells was similar to that of the wild type.

The electron-dense deposits in the chloroplasts and phloem of older mutant leaves appeared to be very similar to the iron core of ferritin, which is known to store iron. Ferritin from iron-loaded *Phaseolus* leaves was isolated, electrophoretically separated, and compared to the iron-

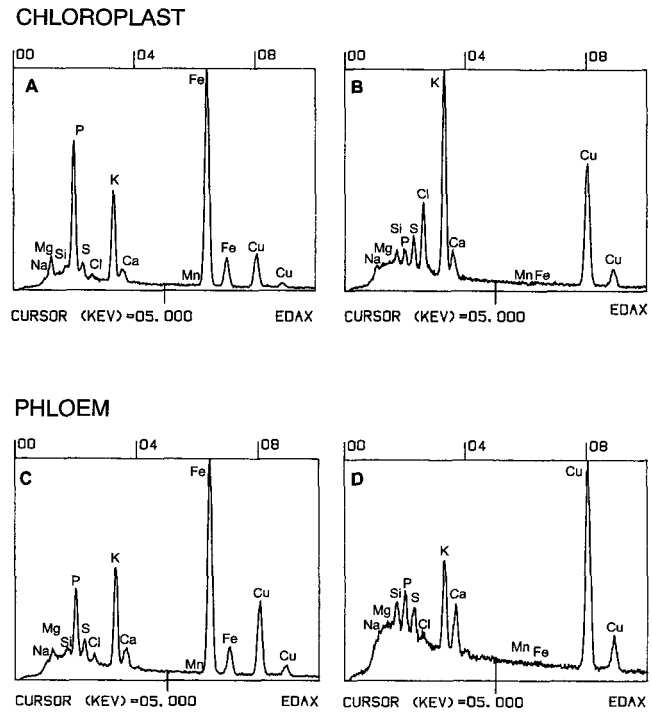

Figure 1. Representative EDAX spectra from electron-dense iron/phosphorus inclusions in chloroplasts of palisade parenchyma (A) and phloem (C) of older mutant leaves. For comparison, spectra of chloroplasts (B) and phloem (D) outside of the electron-dense particles are shown. Leaf sections of 1 μm thickness were analyzed at 6350× magnification. The copper peaks result from copper grid usage.

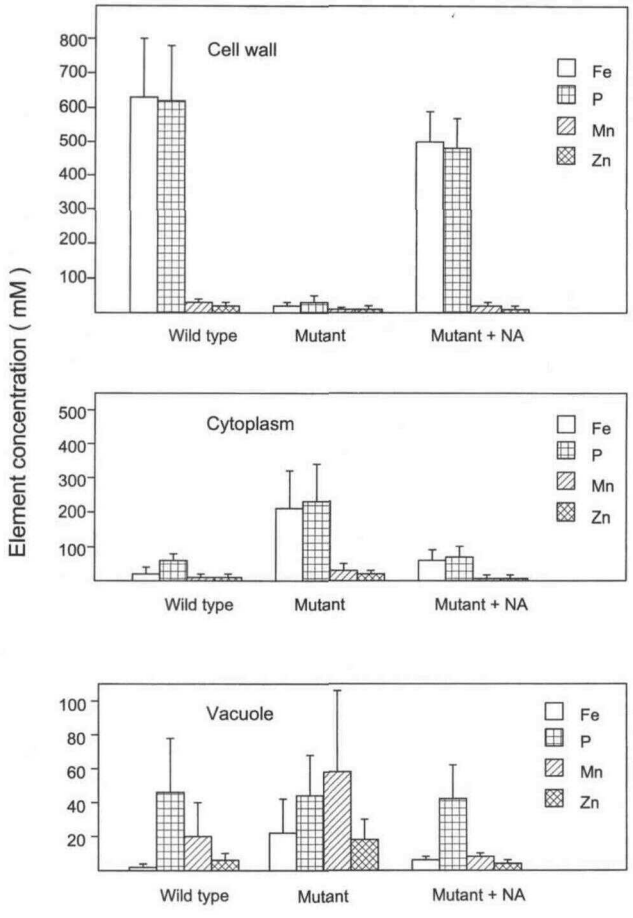

Figure 2. Comparison of element content in rhizodermal cells of roots of wild type, mutant, and mutant + NA by means of x-ray microanalysis in a TEM. Plants were grown in nutrient solution with 10 μM FeEDTA. Mutant plants were treated with 0.5 mM NA via the leaves over a period of 11 d (1 μmol NA per plant in total). Means are presented with SD (n = 5–15).

loaded wild type, control wild type, and mutant *chloronerva* of *Lycopersicon*. Native electrophoresis gels indicated that purified ferritin from iron-loaded *Phaseolus* leaves is essentially pure, with only a single band detected on a Coomassie blue-stained gel (Fig. 3, lanes 2 and 7). The same gel band also stained positive for iron with the Prussian blue reaction (data not shown). The mol wt of *Phaseolus* ferritin was slightly higher than that of horse spleen ferritin. Similar preparations from control bean showed only a very light ferritin band. Iron-loaded wild-type and mutant preparations from tomato actually yielded only very small yellow-brown pellets containing iron and a trace of protein. After PAGE, ferritin protein was not detectable. In contrast, the pellets of iron-loaded *Phaseolus* were red-brown and much larger.

SDS-PAGE of ferritin preparations indicated that high molecular mass *Phaseolus* ferritin consists of two or three subunits (26.5, 25, and 21 kD), whereas horse spleen and poinsettia ferritin revealed only one band, having a lower molecular mass of 19 kD (Fig. 4A, lanes 1 and 9). For immunoblot analyses, antibodies raised against purified *Phaseolus* leaf ferritin were used. As expected, the subunits of iron-loaded (lanes 2 and 7) and control bean ferritin (lane 3) were distinctly recognized by the antibodies (Fig. 4B). An additional subunit (21 kD) was detected after prolonged storage of bean leaf ferritin and is probably a degradation product (Fig. 4B, lane 7). However, neither wild-type tomato nor mutant ferritin preparations were recognized by the ferritin antibodies (Fig. 4B, lanes 4–6). Cross-reactivity of the bean leaf ferritin antiserum was checked with ferritin preparations of other plant species. Strong cross-reactivity was observed with cowpea (Fig. 4B, lane 10) and less with barley (Fig. 4B, lane 8) and poinsettia (Fig. 4B, lane 9). No cross-reactivity could be detected with horse spleen ferritin (Fig. 4B, lane 1). From these results we suggest that ferritin is absent in the tomato mutant *chloronerva*.

To identify the iron/phosphorus deposits in the mutant leaves, we compared the elemental composition of these deposits with the particles of ferritin-containing bean leaves by means of x-ray microanalysis. As a rule, the mutant deposits exhibit higher iron and phosphorus concentrations than *Phaseolus*. Also, the magnesium content is relatively high in the mutant inclusions (Fig. 5). The determination of the iron to phosphorus ratio of the electron-dense particles revealed a ratio of 1 for the mutant to about 4 for the iron-loaded bean. These results indicate that the tomato mutant and the iron-loaded bean leaves possess different types of electron-dense inclusions.

DISCUSSION

Plants have developed several mechanisms to maintain fairly constant internal concentrations of mineral nutrients over a wide range of external concentrations. The negative charges in cell walls of roots act as cation exchangers. Thus, cations can accumulate by a nonmetabolic step in the apoplast of roots. Roots of the tomato wild type accumulated considerable amounts of iron and phosphorus in the cell wall, whereas in the NA-free mutant the accumulation of these elements was negligible. Instead, iron and phosphorus were detected inside the cells (Fig. 2). These differences between the wild type and mutant *chloronerva* are probably due to the strong proton extrusion and enhanced ferric reductase activity in the rhizodermis of the mutant (Stephan and Grün, 1989). The consequence is an increased

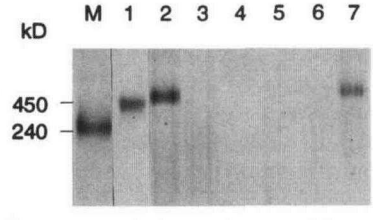

Figure 3. Nondenaturing gel electrophoresis of ferritin from various plants. Purified leaf ferritin preparations of iron-loaded bean (lane 2), control bean (lane 3), iron-loaded tomato wild type (lane 4), control tomato wild type (lane 5), mutant (lane 6), and iron-loaded bean (lane 7). M, Molecular mass standards in kD; lane 1, horse spleen ferritin. The gel was a 5% polyacrylamide gel stained with Coomassie blue R-250.

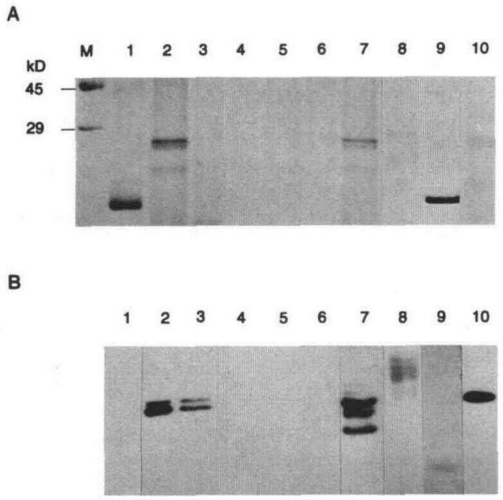

Figure 4. Analysis of ferritin from various plants. A, Coomassie blue R-250 staining of an SDS-polyacrylamide gel (12%) loaded with purified leaf ferritin preparations of iron-loaded bean (lane 2), control bean (lane 3), iron-loaded tomato wild type (lane 4), control tomato wild type (lane 5), tomato mutant (lane 6), iron-loaded bean (lane 7), barley (lane 8), poinsettia (lane 9), and cowpea (lane 10). M, Molecular mass standards in kD; lane 1, horse spleen ferritin. B, Western blot analysis of enriched ferritin preparation from leaves on a 12% polyacrylamide/SDS gel. Separated proteins were transferred to nitrocellulose and labeled with a 1:1000 dilution of bean leaf ferritin antiserum and a 1:3000 dilution of sheep anti-mouse horseradish peroxidase-conjugated IgG. Lanes 1 through 10 are as described in A.

iron uptake and translocation to the shoot of the mutant (Becker et al., 1992; Pich et al., 1994).

X-ray microanalyses of freeze-substituted sections of mutant leaves enabled iron/phosphorus-containing amorphous deposits to be detected in chloroplasts and in phloem (Fig. 1). Since the electron-dense iron cores of ferritin can also be detected by EM (Seckbach, 1982), we tried to characterize the iron/phosphorus-particles in the mutant leaves by comparison with a known plant ferritin as a reference. Plant ferritin is an iron storage protein that accumulates in seeds (Sczekan and Joshi, 1987; Briat et al., 1989), in leaves during senescence (Barton, 1970), or after iron overload (Seckbach, 1972, 1982; van der Mark and van den Briel, 1985). It is located mainly in plastids but also in xylem (Robards and Robinson, 1968) and in phloem (Behnke, 1977). Suggested functions of ferritin are iron storage for intracellular needs (housekeeping ferritin) and protection from toxic effects of iron overload (Theil, 1987). Fe^{2+} may react with reduced forms of O_2 to produce deleterious hydroxyl radicals that accelerate lipid peroxidation (Laulhere and Briat, 1993).

With reference to the high cellular iron concentrations in tomato mutant leaves, ferritin could be a suitable candidate to prevent iron toxicity. However, as shown in Figures 3 and 4A, ferritin could be detected in neither the wild type nor the mutant. Laulhere et al. (1988) investigated the distribution of ferritins in various organs of pea plants. The relative amount of ferritin was variable in the different organs, but in pea leaves ferritin was not detected. Bean leaf ferritin consists of different subunits (Fig. 4), which is in agreement with results of van der Mark and van den Briel (1985) and Korcz and Twardowski (1992). Antibodies raised against bean leaf ferritin recognized not only the polypeptides of its own antigen but also ferritin subunits of the taxonomically nonrelated species cowpea, barley, and poinsettia (Fig. 4B). Therefore, the failure to detect ferritin in mutant leaves cannot be explained by a lack of cross-reactivity with the bean leaf ferritin antiserum. The failure of the antiserum to recognize horse spleen ferritin (Fig. 4B) is consistent with the results from the investigations of Sczekan and Joshi (1987) and Laulhere et al. (1988), which showed that plant ferritins do not share immunological determinants with animal ferritin.

A comparison of iron/phosphorus ratios indicates different types of electron-dense inclusions in iron-loaded bean leaves and in the tomato mutant (Fig. 5). In iron-loaded *Phaseolus* leaves the electron-dense particles represent the iron core of ferritin. It is well known that there are considerable variations in iron/phosphorus ratios of ferritin cores, ranging from nearly 2 in bacterial ferritin to 8 in horse spleen ferritin (de Silva et al., 1993; Wade et al., 1993). However, from the results discussed above, there is no evidence for ferritin depositions in the mutant leaves. Weir et al. (1984) reported that the loss of protein subunits of mammalian hemosiderin can result in the precipitation of the iron complex, which can be seen in electron micrographs. An iron/phosphorus ratio of about 1 in the particles of older mutant leaves could be an indication that the accumulated iron is precipitated as insoluble iron phos-

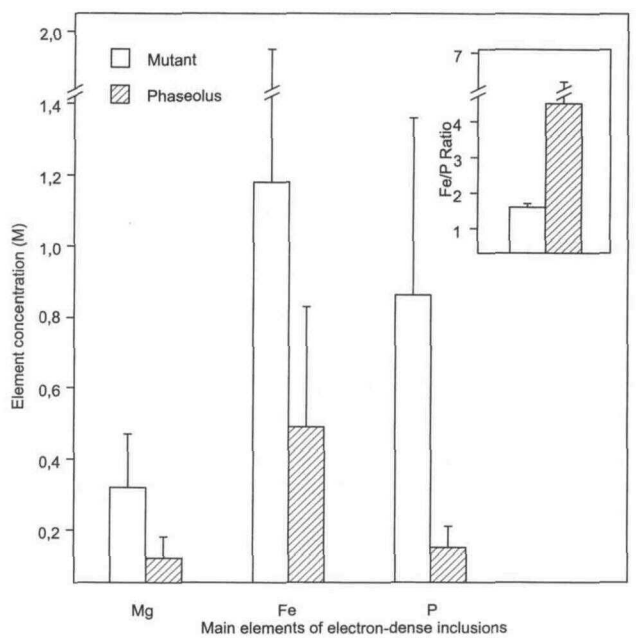

Figure 5. Comparison of element content and iron/phosphorus ratio of electron-dense inclusions in leaves of the tomato mutant and iron-loaded *Phaseolus* based on x-ray microanalysis of 1-μm-thick sections. Values are means + SD of between 21 and 25 determinations. Differences between mutant and iron-loaded *Phaseolus* were significant at $P = 0.001$ (*t* test).

phate or as another iron phosphate compound, for instance phytate. Phytate is considered to function as a reserve of phosphorus and various cations mainly in seeds (Cosgrove, 1980), but it has also been detected in roots (Campbell et al., 1991). The principle minerals stored as phytate are potassium and magnesium (Lott et al., 1982), but also copper, zinc, iron, and calcium (Maga, 1982; Mikuš et al., 1992). Van Steveninck et al. (1990) discuss the binding of excess zinc by phytate as a possible detoxification mechanism, especially in zinc-tolerant plants, but Mössbauer studies of iron in tomato plants showed that the ferric component in tomato seeds is different from those of phytate (Ambe, 1989).

It is known that divalent metals are kept soluble partly by chelation with certain cellular ligands. Because of its ability to form complexes with several divalent metal ions, NA could be predestined to complex ferrous iron in a soluble, nontoxic, and available form. In the absence of NA the excessive iron in the mutant is oxidized to Fe^{3+}. Since the mutant seems to be unable to induce ferritin synthesis under iron excess, the precipitation of insoluble iron compounds could be a mechanism to protect the cell from iron overload. The high concentrations of phosphorus in chloroplasts (12 mM) and in phloem exudate (3–5 mM) and the high pH of the phloem sap (Hocking, 1980; Wade et al., 1993) could favor the precipitation of insoluble ferric phosphate compounds in these compartments. On the other hand, the formation of such insoluble iron compounds decreases the availability of cellular iron and may also prevent the mobility of iron in the phloem transport stream, leading to an insufficient iron delivery to growing apical meristems (Becker et al., 1989). Iron deficiency symptoms of the NA-less mutant *chloronerva* described above could be the consequence of this insufficient iron delivery.

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