Changes in the Iron and Phosphorus Content of Stroma Inclusions during Etioplast-Chloroplast Development in *Nicotiana*

B. Sprey

Institute of Biophysics, Biological Research Center, anlage Jülich

G. Gliem

Institut für Pflanzenkrankheiten der Universität Bonn

and

A. G. S. Jánossy

Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, Szeged

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Conformational changes of the thylakoid arrangement during light-dependent etioplast-chloroplast development in cotyledons of *Nicotiana clevelandii* × *N. glutinosa* are correlated with a decrease of the iron and phosphorus content in electron-dense stroma inclusions. Parallel to the transformation of the prolamellar body and the stacking process of the thylakoids, both the iron and phosphorus content of the inclusions were found to be reduced. Their elemental composition was analysed by means of the energy-dispersive X-ray microanalysis. Due to their high electron-density these stroma inclusions can be observed by conventional transmission electron microscopy in unstained thin-sections from exclusively glutaraldehyde-fixed material. They seem to be involved in membrane formation processes concomitant with the dispersal of the prolamellar bodies. Thus, the iron and phosphorus containing inclusions were found either closely surrounded by membranes or in the intralamellar space of plastids from plantlets illuminated for 1–8 hours. In chloroplasts (illumination period 12–24 hours) no connections between these inclusions and the thylakoids were noticed.

Introduction

In preceding contributions, the occurrence and composition of electron-dense stroma inclusions in proplastids [1], young chloroplasts [2] and plastids of tobacco tumor virus (TTV)-infected plants of *Nicotiana clevelandii* × *N. glutinosa* has been described [3]. Their presence in tobacco chloroplasts as observed by conventional transmission electron microscopy has been documented previously [4–7]. Based on data of the energy-dispersive X-ray microanalysis technique, they were found to contain iron and phosphorus [3]. Both elements are supposed to be covalently bound to a stroma component, since they are not extracted during the dehydration process. In this communication we compare the elemental composition of these stroma inclusions in thin-sectioned etioplasts and chloroplasts of *Nicotiana* by means of X-ray microanalysis.

Requests for reprints should be sent to Dr. B. Sprey, Institut für Botanik und Mikrobiologie der Kernforschungsanlage Jülich, *D-5170 Jülich*.

Material and Methods

Transmission electron microscopy was carried out with a Siemens Elmiskop I. The methods of epoxy-resin embedding and staining procedures were those routinely used for electron microscopy. Elemental analysis was performed using a transmission electron microscope (Jeol 100B) fitted wih a scanning attachment and an energy dispersive X-ray microanalysis system (EDAX). Sample preparation for electron microprobe analysis has been previously described in detail [3, 8]. Seedlings of *Nicotiana clevelandii* × *N. glutinosa* were grown for 3 weeks in darkness on filter paper in Petri dishes. The plantlets were watered with distilled H_2O. They were illuminated for 0.5, 1, 2, 4, 8, 12 and 24 h with 5 klx (Philips TL 40/33, 21 °C). Cotyledons of plantlets continuously illuminated or kept in darkness during the total growth period served as controls. Leaf pieces were fixed either in buffered glutaraldehyde or in glutaraldehyde followed by osmium fixation [3].

Results and Discussion

Etioplasts in *Nicotiana* cotyledons contain electron-dense inclusions (diameter: 0.3–0.1 μm). These consist of a homogeneous opaque center and an irregularly shaped periphery of loosely arranged smaller particles (Fig. 1 *, 4). Because of their high density they can be easily detected in unstained thin-sectioned samples exclusively fixed in buffered glutaraldehyde solution by means of transmission electron microscopy (Fig. 4). For etioplasts (Fig. 1) and etio-chloroplasts illuminated for 1–2 hours (Fig. 2), these stroma inclusions were mostly situated near to the prolamellar body or to the transformed membranes of the prolamellar body.

Illumination of *Nicotiana* cotyledons with white light confirmed the well established structural view

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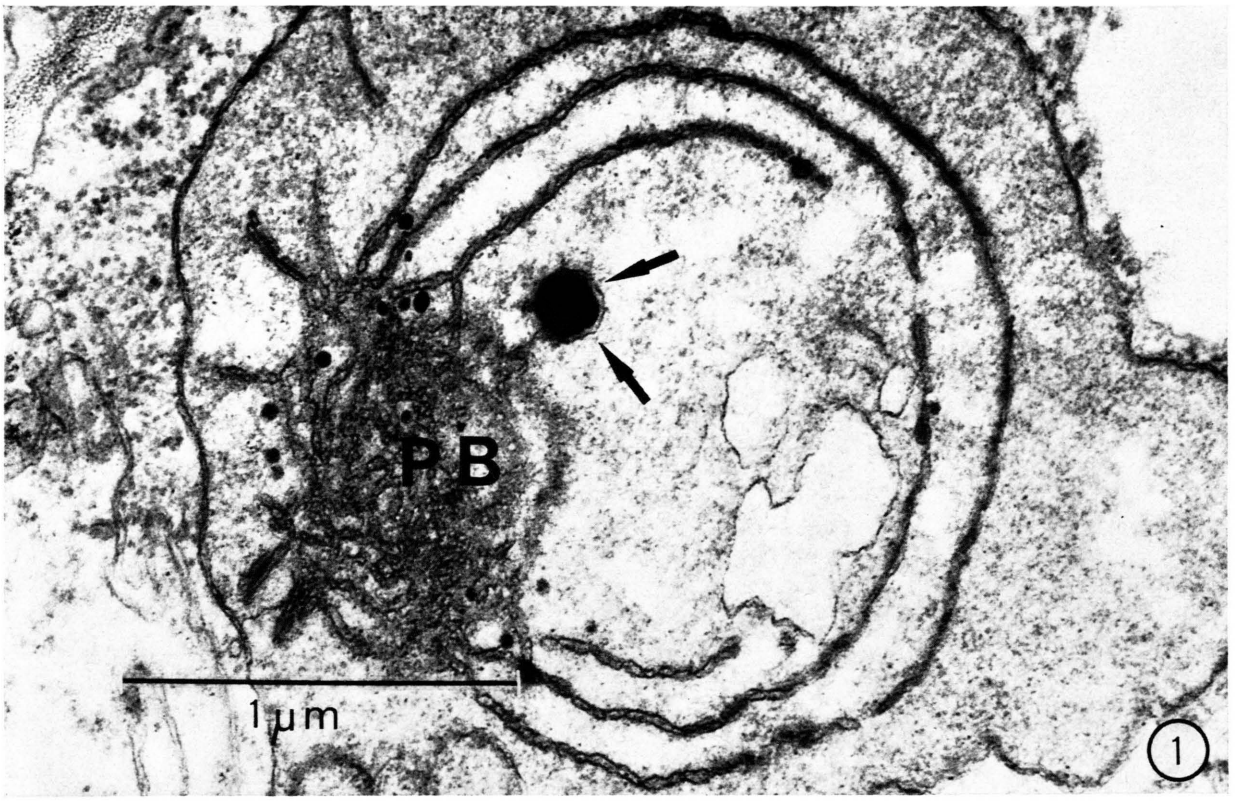
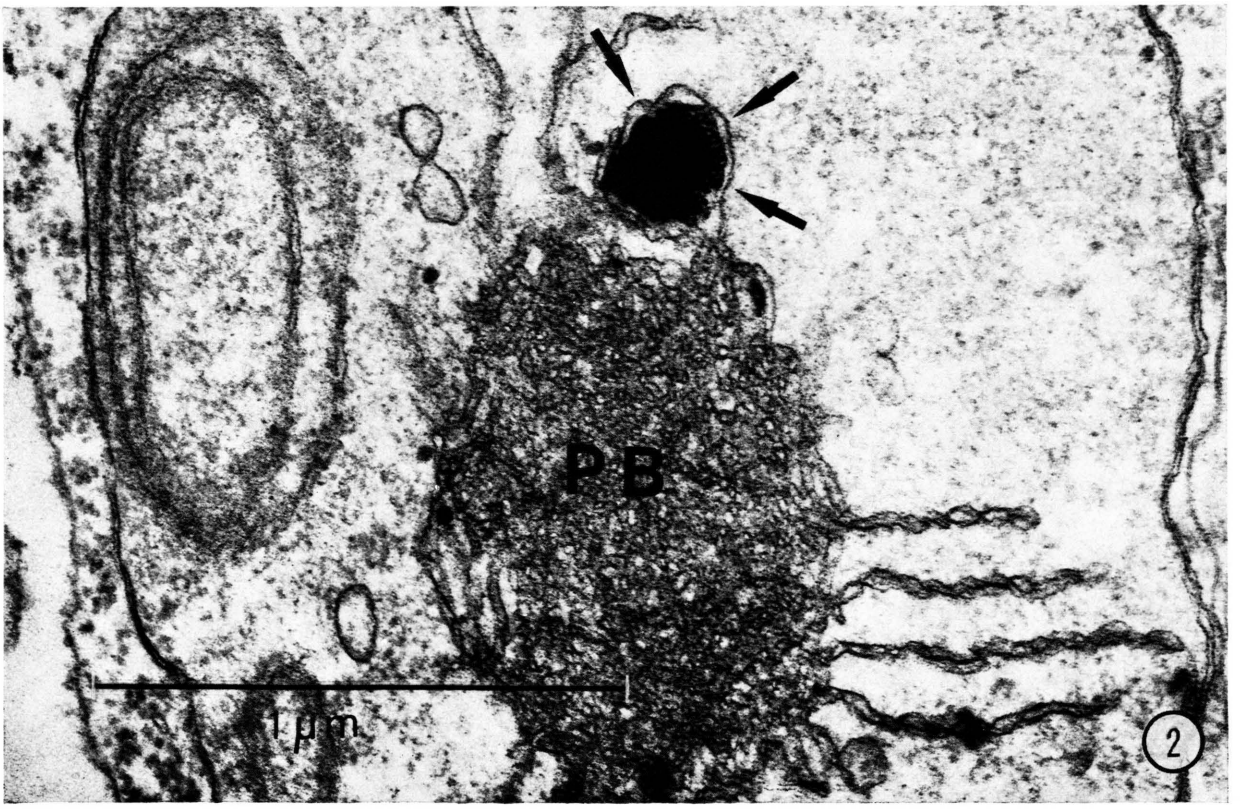
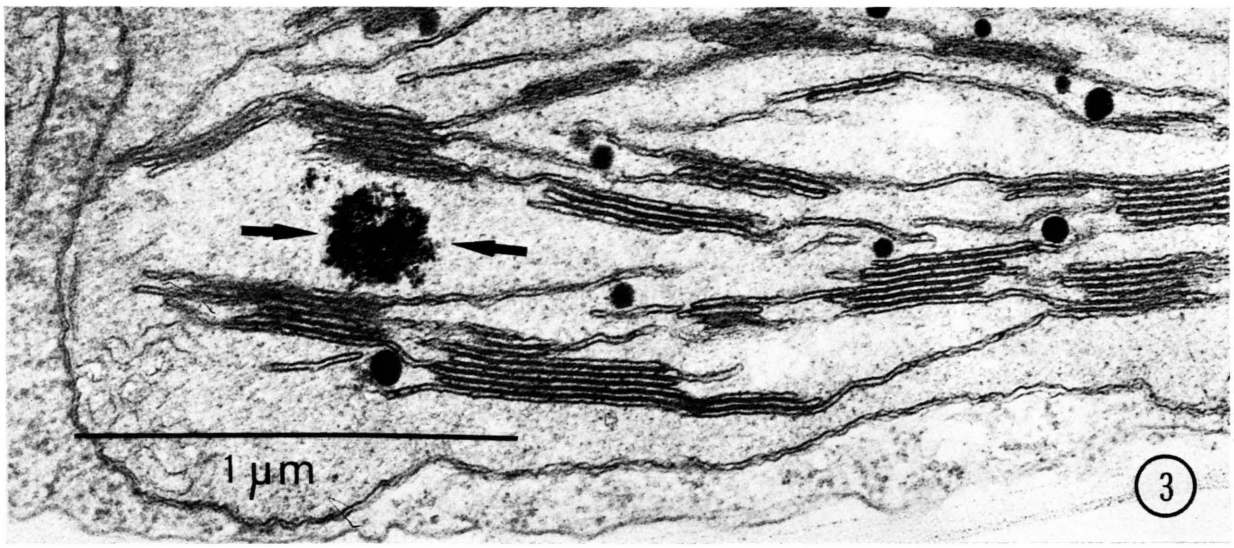
Fig. 1. Etioplast in a *Nicotiana* cotyledon containing a prolamellar body (PB) and an Fe/P-containing inclusion which is partially surrounded by a membrane (arrows). Preparation: Glutaraldehyde-osmium fixation, Epon embedding and lead-citrate staining.

Fig. 2. Plastid in a *Nicotiana* cotyledon after 1 h of illumination. The Fe/P-containing inclusion near to the prolamellar body (PB) appears to be enclosed by a membrane (arrows). Preparation as in Fig. 1.





Legends to Plates on page 138 and 139.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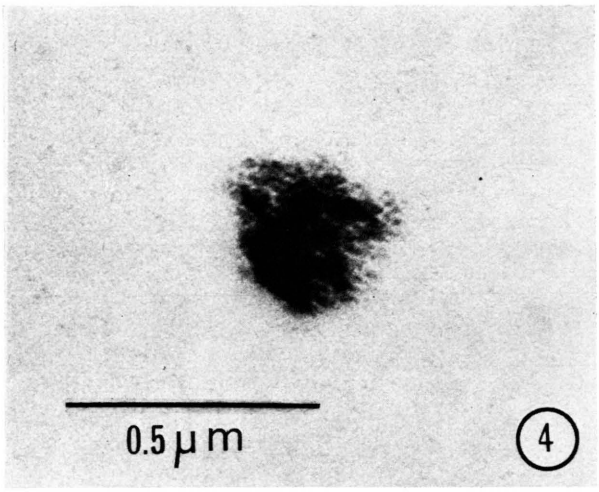
3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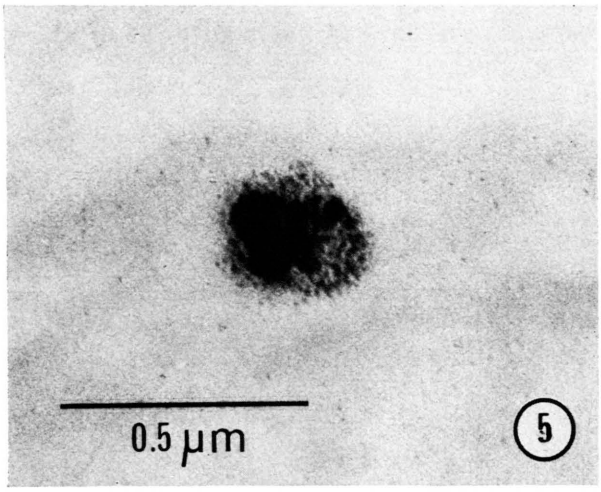
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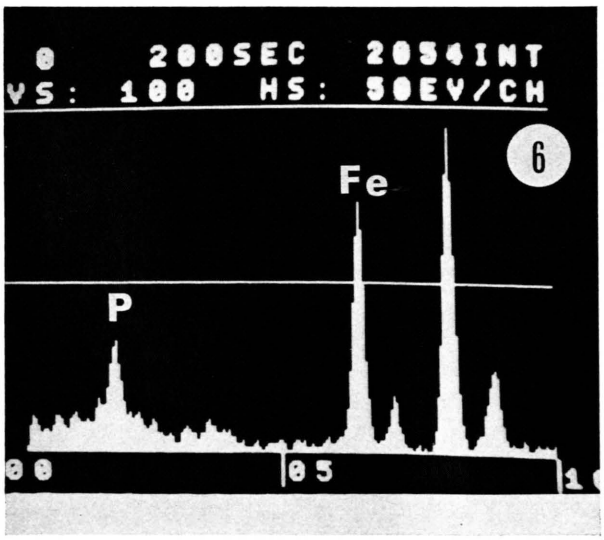
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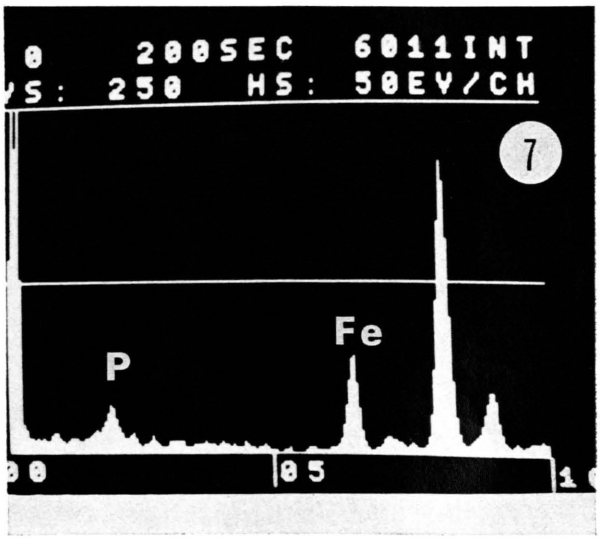
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of the etioplast-chloroplast development [10]. All single steps of structural membrane changes in *Nicotiana* plastids, *e. g.* prolamellar body transformation, stroma lamellae formation and the stacking process of grana lamellae, resembled those commonly accepted for angiosperm plastids [10, 11] (unpublished observations). Fig. 3 shows a chloroplast with an electron-dense inclusion in a *Nicotiana* cotyledon which was continuously illuminated for 24 hours.

During the phase of the prolamellar body transformation and at the beginning of the stacking processes of thylakoids, the electron-dense inclusions were often found near to or directly associated with the thylakoids. Thus, they seem to be enclosed by membranes or apparently exist within the intralamellar space of the thylakoids (Fig. 2). We never noticed a connection between the lamellae and the electron-dense inclusions in chloroplasts with a well developed thylakoid system (Fig. 3 **).

When studied by X-ray microanalysis, the emission spectra from glutaraldehyde-fixed etioplasts in unstained thin-sections (Fig. 4) showed the presence of phosphorus (Kα = 2.00 keV) and iron (Kα = 6.40 keV, Fig. 6). Absolute weight percentages were calculated [9] from background corrected peak counts (mean values from 10 determinations each) yielding 18.9 ± 1.2 wt.% Fe and 2.0 ± 0.4 wt.% P. The corresponding elemental concentrations for electron-dense inclusions from chloroplasts after 24 h of illumination (Figs 5, 7) were 1.41 ± 0.58 wt.% Fe and 0.25 ± 0.11 wt.% P.

Based on the ultrastructural findings and the X-ray microanalysis data it is proposed that the decrease of phosphorus and iron in the electron-dense inclusions is partially related to the process of thylakoid formation during the etioplast-chloroplast development. In contrast to the case of phytoferritin [12], an iron-protein macromolecule often arranged in crystalline order in plastids, we never observed a crystalline substructure in the Fe/P-containing inclusions during etioplast-chloroplast development in *Nicotiana*. A more detailed contribution on the correlation of membrane formation and the electron microprobe analysis data will be reported elsewhere [13].

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* Figs 1—2 see Plate on page 138 a.
** Figs 3—7 see Plate on page 138 b.

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Fig. 3. Chloroplast in a *Nicotiana* cotyledon after an illumination period of 24 h. The Fe/P-containing inclusion (arrows) shows no connection to the lamellae. Preparation as in Fig. 1.

Fig. 4, 5. Electron-dense Fe/P-inclusions present in unstained thin-sections of an etioplast (Fig. 4) and a chloroplast in *Nicotiana* cotyledons after an illumination period of 24 h (Fig. 5).

Fig. 6, 7. X-ray emission spectra from glutaraldehyde-fixed, unstained inclusions of an etioplast (Fig. 6) and a chloroplast from a *Nicotiana* cotyledon illuminated for 24 h (Fig. 7). The spectra show the presence of phosphorus and iron. Additional peaks indicate the presence of Kα and Kβ-lines of copper, derived from the supporting grid.

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