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PHYTOFERRITIN IN PLASTIDS OF BLACKBERRY LEAVES

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Introduction

Ferritin is an iron-containing protein present in the cells of many plants and animals. Plant ferritin, the so-called phytoferritin, was first discovered by Hyde et al. (1963). Since then phytoferritin has been observed by several workers in various plants (e. g. Barton 1966, 1970, Robards and Humpherson 1967, Robards and Robinson 1968, Seckbach 1968, 1969, 1972, Amelunxen et al. 1970, Behnke 1971, Mesquita 1971/72, Maramorosch and Hiraumi 1973 etc.).

Phytoferritin is almost exclusively located in the plastid stroma (except the ferritin in fungi — Peat and Banbury 1968) in contrast to other ferritins, which were observed in several cell organelles and in the cytoplasm. The arrangement of the phytoferritin particles in thin sections is crystalline, paracrystalline, or irregular. Each phytoferritin granule has an electron-opaque micelar core (5.5 — 6 nm in diameter). The electron-opaque core contains about 5000 iron atoms (Fischbach and Anderegg 1965).

Many authors supposed that within the life span of plastids the phytoferritin particles function as a storage material for iron in a non-toxic form. According to this opinion the iron from the phytoferritin can be utilized for the formation of the photosynthetic apparatus. An attempt to prove this possibility is described in this paper.

Material and Methods

Blackberry plants (*Rubus fruticosus* L. s. l.) were grown in natural conditions. The regreening of plastids in yellow (senescent) leaves was studied in leaf discs (16 mm in diameter). The leaf discs were placed on filterpaper in petri-dishes. The discs were cultured under a 16 hours

photoperiod (fluorescent tube, "Flora", TT, Zagreb, Yugoslavia) during a period of several weeks. The filter-paper was wetted with solution of sucrose (0.1 M), kinetin (0.001 %) or gibberellic acid (0.05 %) in tap water. The solutions were changed each 3 days.

For electron microscopic investigation the material was fixed in 0.5 % glutaraldehyde in cacodylate buffer at pH 7.2 for 30 minutes. After fixation the material was washed in cacodylate buffer (1 — 2 hours) and postfixed in 1 % OsO_4 (2 hours). In some cases the postfixation was omitted. The fixed material was dehydrated in ethanol series. All these preparations were performed at a temperature of about 1 °C. After the dehydration the material was embedded in Araldite Cargille 6005. Ultrathin sections were made with glass knives on a Reichert Om U2 ultramicrotome, and stained with uranyl acetate and lead citrate (sometimes these stainings were omitted). The sections were examined with a Siemens Elmiskop I.

The pigments were extracted by grinding the leaves with a small amount of $MgCO_3$ and quartz sand in 80 % acetone. After centrifugation the supernatant was decanted and pellet re-extracted several times with 80 % acetone. The quantitative determination of the chlorophyll was performed at 663 and 645 nm (Holden 1965) on a Beckman DBGT spectrophotometer.

Results

In fully expanded green leaves of blackberry the mesophyll chloroplasts are normal in structure (Ljubešić 1970) and usually without phytoferritin particles (Fig. 1). Very rarely such chloroplasts may contain small phytoferritin inclusions.

Phytoferritin particles were observed, however, in some plastids of green leaves. In the cells close to conductive tissue the plastids are always undeveloped. The thylakoid system of these plastids is poor. Normal grana do not exist and only several single thylakoids are present. Among the thlakoids there occur numerous and relatively big plastoglobules. Near the border of some of these plastids usually occur big crystalline or paracrystalline accumulations of phytoferritin (Fig. 2). The average size of such aggregations is ca. 0.2 μm in diameter. In crystalline inclusions the distance between two neighbouring particles is about 10 nm (center

Fig. 1. The chloroplast from a normal green leaf. 60,000 : 1.

Fig. 2. Part of a plastid from a cell close to conductive tissue. Inclusion of phytoferritin is present. The plastid contans only a few single thylakoids and some big plastoglobules. 66,000 : 1.

Fig. 3. Phytoferritin accumulation in plastid of old leaf. The material was prepared without OsO_4-postfixation. Unstained section. 66,000 : 1.

Fig. 4. Portion of a plastid of an old yellow-green leaf. Besides partially preserved grana the plastid contains a starch grain and a big phytoferritin aggregation. 70,000 : 1.

Fig. 5. Plastid of an old yellow leaf. Only numerous big plastoglobules and a phytoferritin inclusion are present. 70,000 : 1.

Fig. 6. Chloroplast of a regreened leaf disc (after 21 days of kinetin treatment). The thylakoid system is normal. Big plastoglobules and starch grains are present. 22,800 : 1.

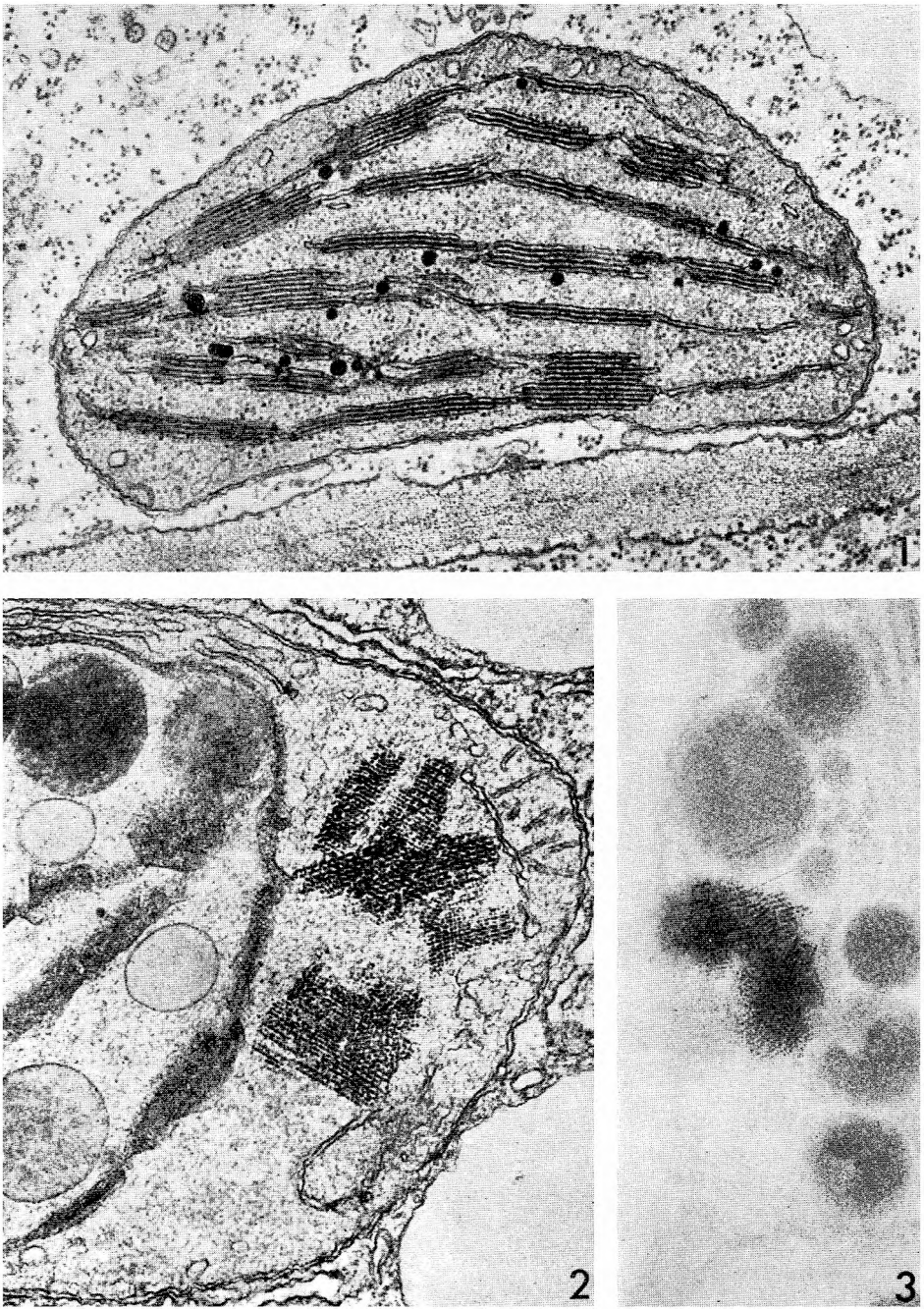
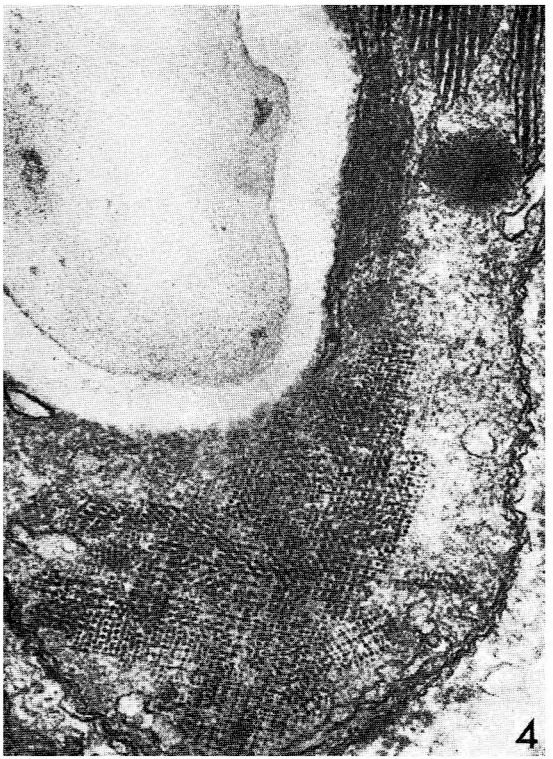


Fig. 1—3.



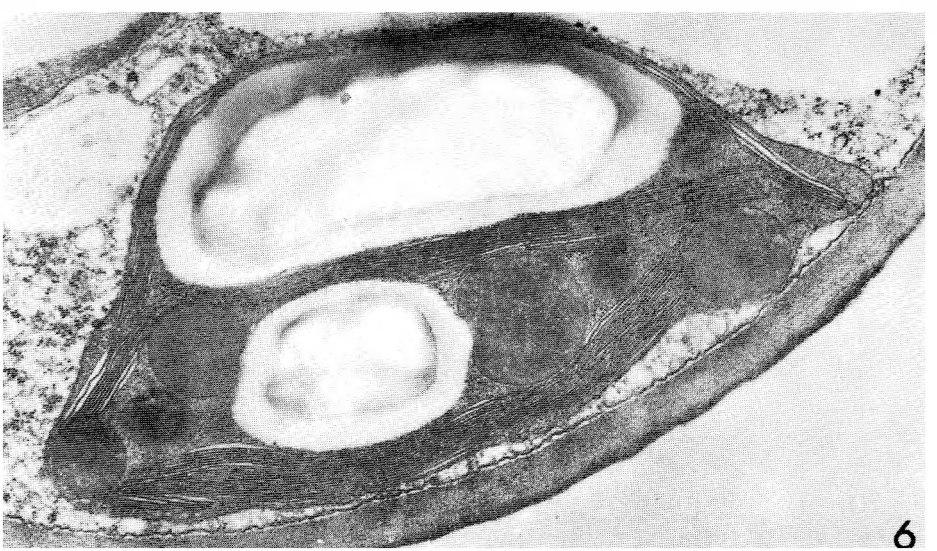


Fig. 4—6.

to center spacing). Single particles or irregularly arranged clusters of phytoferritin were never observed.

However, similar phytoferritin inclusions occur in plastids of leaves which just started to senescence (Fig. 4). At the beginning these inclusions are small and not numerous. But parallelly with the process of senescence the amount and size of phytoferritin aggregations rise. Because the thylakoid system is partially preserved, in such plastids the crystalline inclusions of phytoferritin occur beside grana. A sign of ageing of these plastids is also the presence of starch grains which gradually disappear (Fig. 4).

At a later stage of senescence the plastids show a marked decrease in size, they completely lose their thylakoids and simultaneously accumulate numerous big plastglobules and some phytoferritin inclusions (Fig. 5).

To prove that these particles represent phytoferritin the material was fixed in glutaraldehyde, but without OsO_4-postfixation. Thin sections were not stained with heavy metals either. In spite of the absence of contrast in all cell structures, the iron-rich cores of phytoferritin particles is clearly visible as dark dot-like structures (Fig. 3).

In earlier stages of senescence the leaves are yellow-green and contain less than half of the chlorophyll content of normal green leaves (Table 1). The thylakoid system is partially preserved (Fig. 4). The stroma is here practically without ribosomes but with a large phytoferritin aggregation and with numerous plastoglobules. Such plastids could be regreened with a treatment by kinetin (Table 1). The treatment with

Table 1. The effect of kinetin on the chlorophyll content in the disc of yellow-green leaves.

Incubation period (days)	Total chlorophyll ($mg \cdot cm^{-2}$ of leaf surface)
0	0.016
7	0.022
14	0.033
21	0.035

gibberellic acid had a similar effect. The process of regreening was carried out on leaf discs. After two or three weeks treatment with kinetin or gibberellic acid the yellow-green discs became green. The amount of chlorophyll rises but it does not reach the value of normal green leaves (about $0.04\ mg \cdot cm^{-2}$ of leaf surface). There are also some visible changes on the ultrastructural level (Fig. 6). The stroma becomes rich with ribosomes, some new thylakoids are rebuilt, and all chloroplasts contain big starch grains and few large plastoglobules. During the process of thylakoid rebuilding the accumulations of phytoferritin disappear. It is extremly rare that regreened chloroplasts contain small aggregations of phytoferritin.

Discussion

The appearance of phytoferritin in plastids during the senescence of leaves has been several times described (e. g. Barton 1966, 1970, Catesson 1970). These authors believe that phytoferritin particles represent a product of thylakoid desintegration. Observations described in this paper are in accordance with this opinion. Chloroplasts with a normal intact thylakoid system never contain phytoferritin. But simultaneously with thylakoid desintegration phytoferritin appears in the stroma of many aged plastids. In fact, during the desintegration of thylakoids the surplus of iron, which the plant stores in non-toxic form, appears as phytoferritin particles.

In plastids which are located close to conductive tissue the situation is just opposite. In these plastids a normal thylakoid system does not exist nor has it existed. The phytoferritin represents here the surplus of iron which has never been built into the photosynthetic apparatus.

Behnke (1971) found phytoferritin in sive tube plastids, which are completely without thylakoids. He thinks that the formation of phytoferritin in sive-tube plastids may help to equalize the concentration of iron when its content is raised, e. g. in autumn, as it was formally assumed for ferritin in phloem-parenchima plastids (Catesson 1966).

The disappearance of phytoferritin during the process of regreening of plastids seems to be a proof that phytoferritin represents a storage of iron in a non-toxic form which serves as an iron pool for the active photosynthetic apparatus.

It seems thus that there are some similarities between phytoferritin and plastoglobules. The appearance of both inclusions is correlated with the desintegration of thylakoids. The plastoglobules also partially disappear in the process of the rebuilding of thylakoids during the regreening of chromoplasts (Ljubešić 1968, Devidé and Ljubešić 1974), i. e. the material from plastoglobules is used for the building of new thylakoids. The results of this work thus indicate that the material from phytoferritin inclusions is used in the process of rebuilding the thylakoids, the photosynthetic apparatus respectively.

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Summary

Crystalline inclusions of phytoferritin particles occur within the stroma of mesophyll plastids of senescing blackberry leaves (*Rubus fruticosus* L. s. l.). The regreening of the senescing leaves is correlated with the disappearance of these phytoferritin inclusions.

The origin and function of phytoferritin particles in plastids, especially during the process of regreening of blackberry plastids, are discussed.

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SADRŽAJ

FITOFERITIN U PLASTIDIMA LISTOVA KUPINE

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U ostarjelim listovima kupine (*Rubus fruticosus* L. s. l.) u plastidima mezofila nalaze se nakupine fitoferitina. Ozelenjavanje ostarjelih listova uzrokuje nestajanje fitoferitinskih uklopina iz strome plastida.

Diskutirani su porijeklo i uloga fitoferitina u plastidima kupine, posebno tijekom procesa ozelenjavanja listova.

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