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INTRATHYLAKOIDAL PROTEIN CRYSTALLOIDS IN SPINACH PLASTIDS

Mit deutscher und kroatischer Zusammenfassung
Sa sadržajem na njemačkom i hrvatskom jeziku

MERCEDES WRISCHER

(Laboratory of Electron Microscopy, Institute »Ruđer Bošković«, Zagreb)

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Electron microscopic investigations of spinach leaves fixed during the winter season repeatedly revealed crystalline inclusions in the intrathylakoidal space of leaf plastids, which will be described here in detail.

Material and Methods

Pieces of spinach leaves *(Spinacia oleracea,* various cv.) were fixed in 1% glutaraldehyde, postfixed in 1% OsO_4 and embedded in araldite. Ultrathin sections were cut with a Reichert Ultramikrotom OmU2, stained with uranyl acetate and lead citrate (Reynolds 1963) and examined in a Siemens Elmiskop I (at the Institute of Biology, University of Zagreb).

Thin, hand made sections through the same leaf tissue — after a short fixation in glutaraldehyde — were stained with mercuric bromophenol blue (Mazia et al. 1953) and examined with a light microscope.

Results

Yellow, yellow-green and dark green (i. e. fully grown) spinach leaves were investigated. Characteristic inclusions in plastids were found in all leaves, although their number was much higher in young (yellow or yellow-green) leaves, than in the fully grown leaves. The fine structure of chloroplasts from the dark green leaves is, except for the inclusions, more or less normal and will not be described here.

The plastids from the yellow or yellow-green leaves are, on the other hand, quite different. They are usually round or of a amoeboidal

shape. In plastid stroma one or several prolamellar bodies, mostly of a regular crystal-lattice-like structure, may be observed (Fig. 1, 5). Some thylakoids arise from the prolamellar bodies. Small grana may be developed on them (Fig. 1, 4). Many saccules protrude from the inner part of the outer membrane into the stroma (Fig. 1, 5) in which ribosomes are abundant. Starch grains are also present (Fig. 1). In addition to those, in sections through each plastid, one or several characteristic inclusions can be seen (Fig. 1). These inclusions appear in sections usually triangular or hexagonal and are always bounded by a membrane (Fig. 1, 2, 3, 4, 7, 8). The content of such inclusions is finely granular. Usually the granules (measuring about 5 nm in diameter) are arranged in parallel lines at a distance of 10 nm (Fig. 2). Sometimes another striation can be seen running more or less perpendicularly to the first plane of symmetry (Fig. 3, 4). The contrast of the granules is low even after double staining. As said before, these inclusions are clearly limited by a membrane. In many places a direct communication between such inclusions and the thylakoidal spaces can be seen (Fig. 7, 8), so that the inclusions must be interpreted as dilated parts of the thylakoid system. Sometimes, especially in very young plastids, the ends of the thylakoids are swollen, forming small vesicles which are filled with a dark substance (Fig. 5). Some of the vesicles begin to turn angular, which probably means that the crystallisation of the content has just begun. The next step could be seen in figure 6, where the particles are already arranged in a regular way, forming small striated inclusions.

Explanation of figures

Fig. 1. Plastid from a yellow spinach leaf. A big prolamellar body (pb), thylakoids (t), small grana (g), starch grains (s) and several crystalline inclusions (i) are visible. 45 000 : 1.

Fig. 2. Portion of the plastid from Fig. 1. Higher magnification shows two crystalline inclusions. 80 000 : 1.

Fig. 3. Portion of a plastid from the yellow-green region of a spinach leaf containing a crystalline inclusion which is clearly bounded by a membrane. 100 000 : 1.

Fig. 4. The same as in Fig. 3. 100 000 : 1.

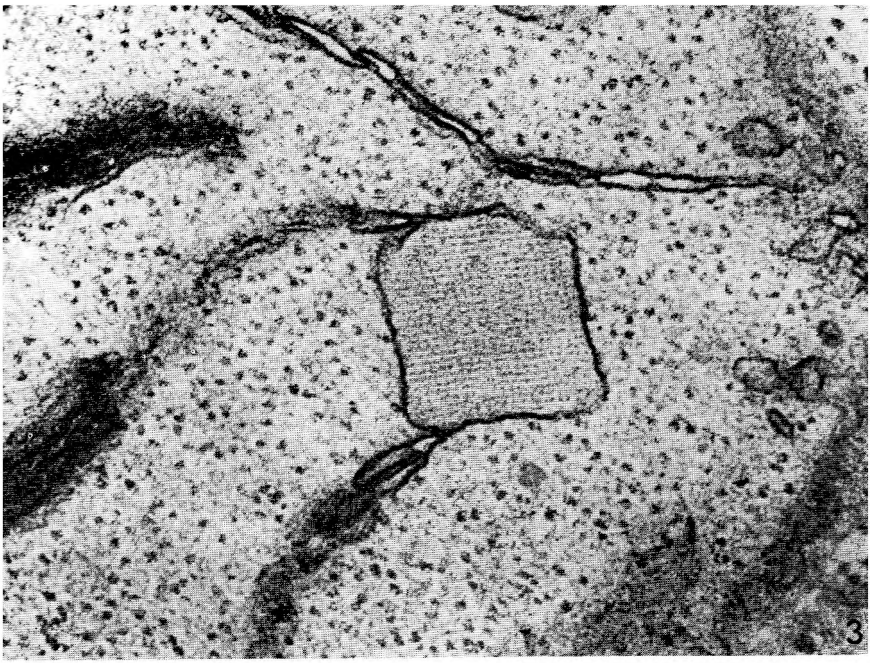
Fig. 5. Plastid from a yellow spinach leaf, containing a prolamellar body (pb) and dilated ends of the thylakoids with dark content (↑). 50 000 : 1.

Fig. 6. Portion of a plastid from a yellow spinach leaf. Thylakoids protrude from the prolamellar body (pb), their dilated ends forming crystalline inclusions (↑). 80 000 : 1.

Fig. 7. Crystalline inclusion in the plastid of a yellow spinach leaf. The inclusion is clearly continuous with the intrathylakoidal space (↑) 80 000 : 1.

Fig. 8. The same as in Fig. 7 (↑). 100 000 · 1.

Fig. 1—2.



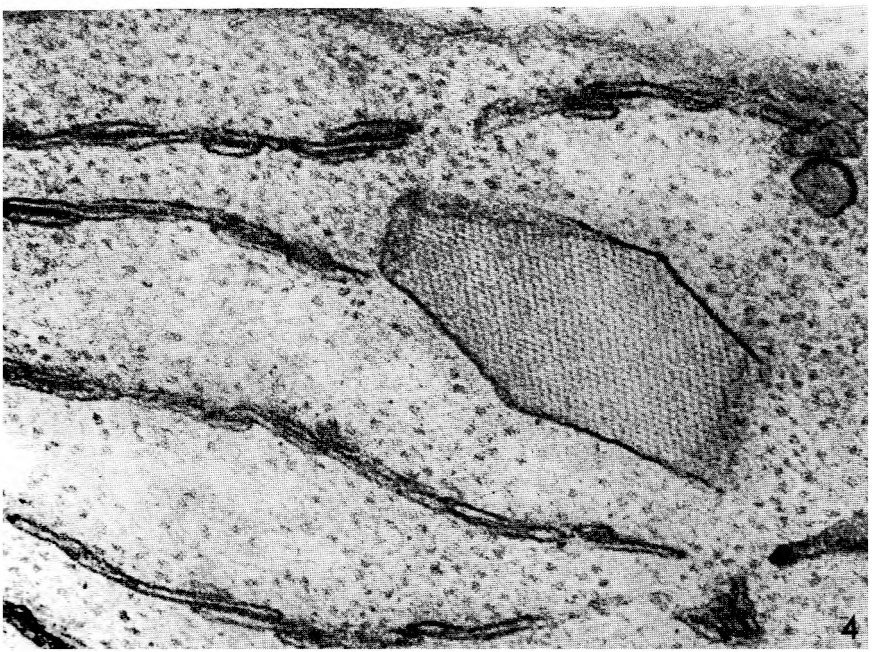
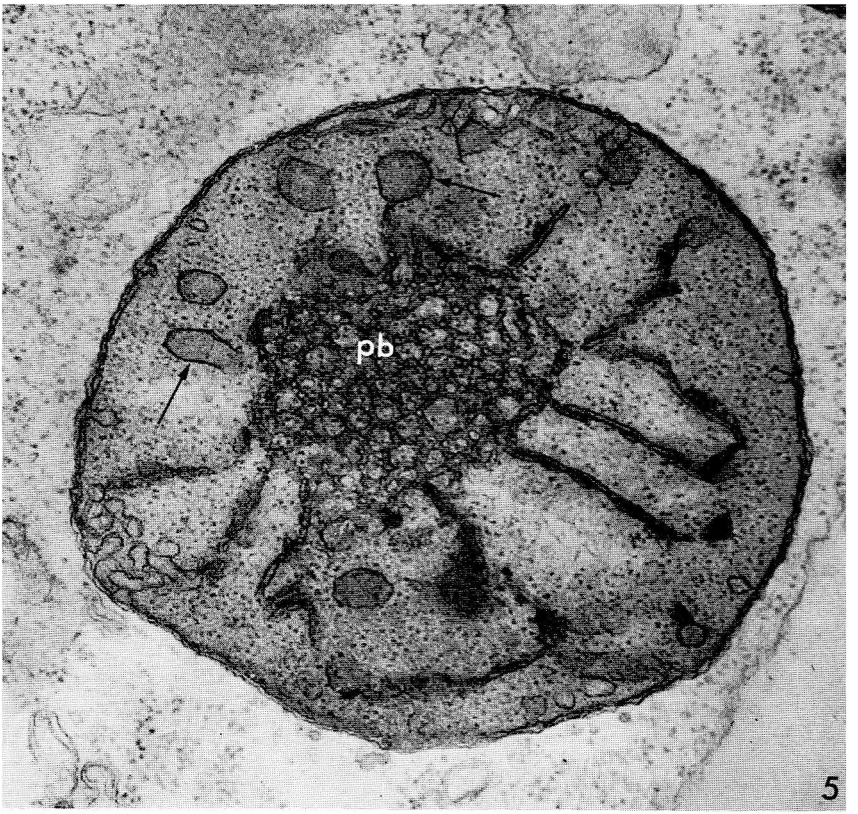


Fig. 3—4.



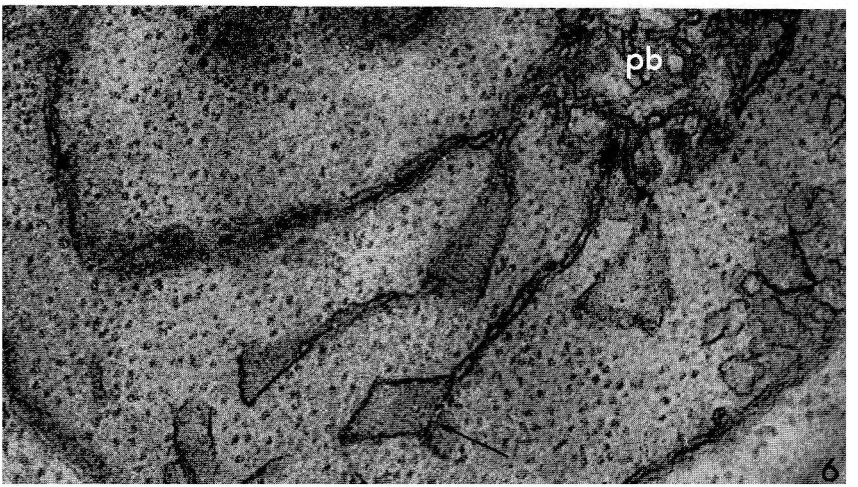


Fig. 5—6.

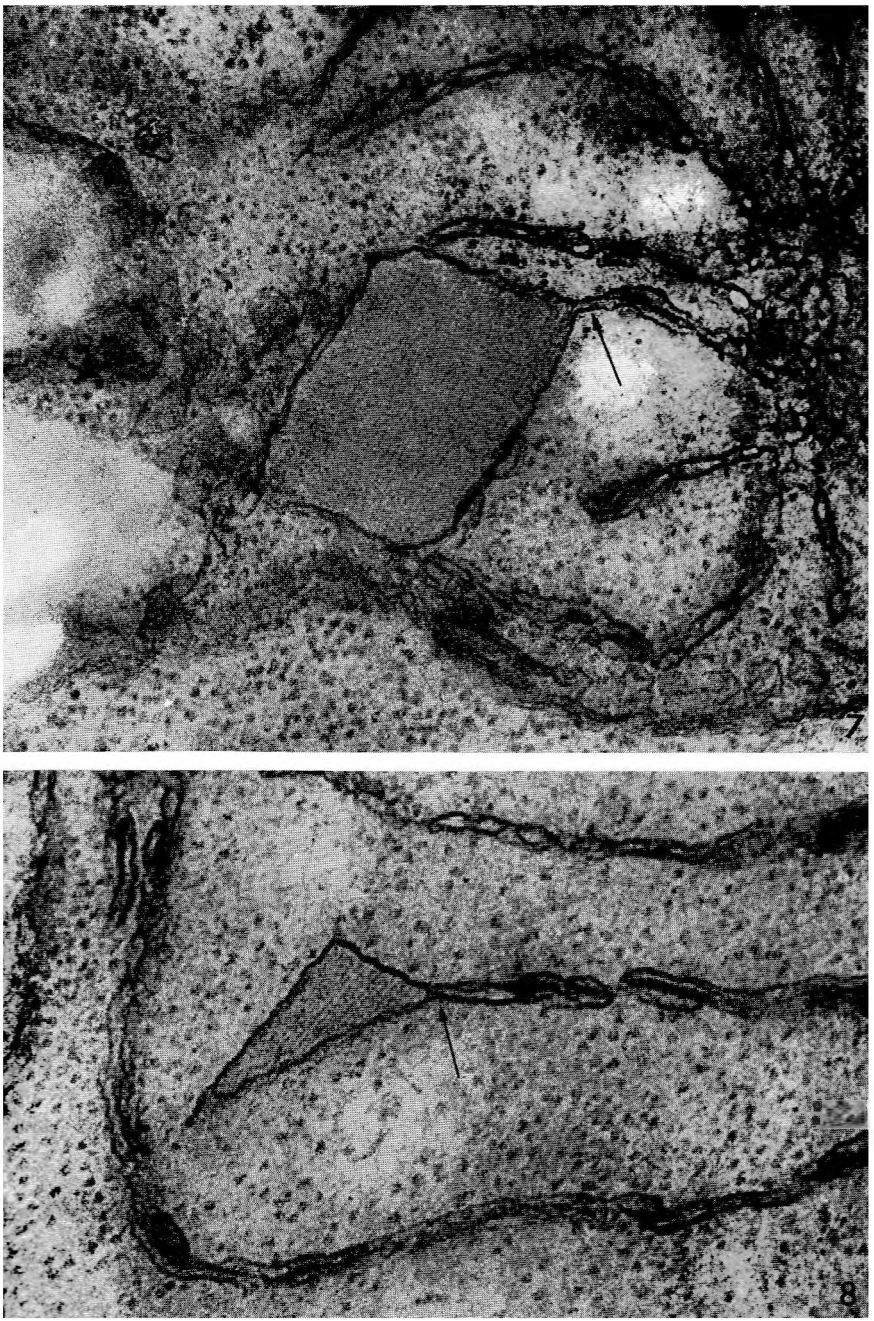


Fig. 7—8.

On hand cut sections through the leaf tissue some minute particles can be seen in the light microscope — one or more in each plastid — which stain intensely with mercuric bromophenol blue. This indicates that such particles may be of proteinaceus nature. As their dimensions and form are in accordance with the dimensions of the crystalloid inclusions found in electron microscope, they appear to be identical.

Discussion

The prolamellar bodies, observed in the yellow or yellow-green spinach leaves, seem to be the so called »weak-light« prolamellar bodies, which could be experimentally obtained in young bean leaves when the plants were kept in weak light (Wrischer 1966). Like the prolamellar bodies from bean leaves, the prolamellar bodies found in spinach plastids disappear after a shorter illumination with strong light of several thousand lux.

Protein inclusions found in plastid stroma have been described several times, as in bean leaves (Lemoine 1966, Dolzmann and Ullrich 1966 and others) and in isolated spinach (Perner 1963) and horse-bean plastids (Shumway et. al. 1967). Their proteinaceus nature was proved by successful digestion on ultrathin sections with pepsin (Wrischer 1967). These inclusions lie free in the stroma and are never bounded by a membrane. In some cases indeed a membrane enveloping the crystalloids has been described (Price et al. 1966, Behnke 1969).

On the other hand, plastids described here for spinach leaves, resemble very much the protein-storing leucoplasts found by Newcomb (1967) in bean root tips. As in bean root tips, the inclusions in spinach leaves could also be stained with mercuric bromophenol blue. This indicates that they contain proteins. But while the thylakoid system in root leucoplasts is very rudimentary, in plastids from the yellow or yellow-green spinach leaves small grana also occur in addition to prolamellar bodies, so that these plastids should be classified as a transitive stage between etioplasts and chloroplasts.

Newcomb (1967) considers the protein inclusions in root leucoplasts as protein storing places. Whether or not the same could be stated also for the inclusions found in spinach leaf plastids, or whether other factors (e. g. low temperature) could play a certain role in their formation is unknown and requires further experimental work.

Summary

Yellow, yellow-green and green spinach leaves contain plastids with characteristic intrathylakoidal protein inclusions. The fine structure of these plastids, as well as protein inclusions have been described.

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ZUSAMMENFASSUNG

INTRATHYLAKOIDALE EIWEISS-KRISTALLOIDE IN DEN PLASTIDEN VON SPINAT

Mercedes Wrischer

(Institut »Ruđer Bošković«, Zagreb)

Gelbe, gelb-grüne und grüne Spinatblätter enthalten Plastiden mit charakteristischen intrathylakoidalen Proteineinschlüssen. Der Feinbau dieser Plastiden sowie ihrer Proteineinschlüsse wurde beschrieben und diskutiert.

SADRŽAJ

INTRATILAKOIDNI PROTEINSKI KRISTALOIDI U PLASTIDIMA ŠPINATA

Mercedes Wrischer

(Institut »Ruđer Bošković«, Zagreb)

Žuti, žuto-zeleni i zeleni listovi špinata sadrže plastide s karakterističnim intratilakoidnim uklopinama. Opisana i diskutirana je fina građa tih plastida kao i njihovih proteinskih uklopina.

Dr Mercedes Wrischer,
Institut »Ruđer Bošković«
Bijenička 54
Zagreb (Jugoslavija)