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Crystals and fibrils in chromoplast plastoglobules of *Solanum capsicastrum* fruit

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The fine structure of the fibrils and crystals, associated with the chromoplast plastoglobules of ripening *Solanum capsicastrum* fruit, was investigated: (1) in thin sections of fixed tissue and (2) in isolates prepared from fresh tissue, subjected to negative staining and metal shadowing. Electron micrographs of thin sections, particularly when combined with TCH-Ag-proteinate staining, afforded particularly clear insights into the ultrastructure of the fibrils and their formation next to the plastoglobules, while the crystals were seen only in transverse section. In the negatively stained isolates, on the other hand, the crystals were well visible as elongated platelets, which could be as short as 1 μm and only about 1.5 nm thick. They were occasionally aggregated into clusters with irregular outlines, about 2–3 μm in diameter. The crystals likely contained carotenoids and their structure was comparable to the large carotene crystals found in other plants.

Key words: *Solanum capsicastrum*, fruit, cromoplast, plastoglobule, crystal, fibril

Introduction

Plastoglobules are lipid-containing spherical bodies present in all types of plastids and are considered to serve as a depot of solubilized carotenoids. Inside the plastoglobule, a peripheral lipoprotein layer tends to envelope a fluid, non-organized interior. Recently, a number of additional plastoglobule proteins were identified; their composition depends on the plastid type (YTTERBERG et al. 2006).

In a previous paper, we presented the ultrastructural and functional changes in the chromoplasts of ripening *Solanum capsicastrum* fruit (LJUBEŠIĆ et al. 2001). Here we focus on two substructures that develop in chromoplast plastoglobules. Electron micrographs of thin sections of fixed and embedded tissue are compared with those of isolates prepared from fresh tissue which were subjected to negative staining and metal shadowing.

Materials and methods

Plants of *Solanum capsicastrum* Link. were grown under greenhouse conditions. For ultrastructural analysis, pieces of tissue of different developmental stages were fixed in 1%

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glutaraldehyde (30 min), rinsed, and postfixed in 1% OsO_4 (2 h); all solutions were prepared in, and the rinsing was performed with, 0.05 M sodium cacodylate buffer (pH 7.2). After dehydration, the material was embedded in Spurr's medium. Sections were prepared and stained with uranyl acetate and lead citrate and examined using a Zeiss 10 electron microscope. For the visualisation of glycolipids, deosmicated sections were subjected to the thiocarbohydrazide-Ag proteinate procedure (TCH-Ag-proteinate-staining) (WRISCHER et al. 2001).

For the preparation of isolates, fresh fruit tissue was squashed in 10 ml of 0.05 M K-Na-phosphate buffer (pH 7.2) or in distilled water. After filtering through flannel cloth to remove coarse particles, the suspension was negatively stained with 2% phosphotungstic acid or shadowed with palladium.

Results

Young green fruit of *S. capsicastrum* contained small chloroplasts with grana of 3–5 thylakoids and few small plastoglobules (LJUBEŠIĆ et al. 2001). During fruit ripening the number of thylakoids was reduced, while the number and the dimensions of the plastoglobules drastically increased. In the large plastoglobules (0.1–0.2 μm in diameter) of orange (half-ripe) fruit two types of inclusions appeared. One type, in its initial stage, appeared as a lightly stained zone which gradually increased in size and assumed a regular shape resembling a crystal. At the same time the osmiophilic (dark) portions of the respective plastoglobule were reduced in size. The crystals appeared to be thin plates, 10–30 nm thick and about 0.2–0.4 μm long (Fig. 2). Due to their thinness, they were mostly cut transversely. According to their low contrast, the crystals obviously contained components that were dissolved during tissue preparation (dehydration). In the chromoplasts of ripe red fruit the plastoglobules almost completely disappeared, and the regular outlines of crystals seen in transverse section, enveloped only by an osmiophilic membrane, became well visible (Fig. 3). The crystals were here and there aggregated into groups.

At the same time, in the chromoplasts of orange and red fruit, another structure appeared in contact with the plastoglobules. These structures were thin long, straight or slightly bent fibrils. They were roundish in cross section and 20–25 nm in diameter and often appeared together with crystals, in the same chromoplast, although in different plastoglobules. The contact of the fibrils with plastoglobules was well visible particularly after TCH-Ag proteinate staining, which is specific for glycolipids (Fig.1). When cut transversely, a thin, dark, finely granular, ring became visible at the periphery of the fibrils, indicating the presence of lipids. The core of the fibrils was lightly stained, and obviously contained substances that were dissolved during preparation. As fruit ripening proceeded the osmiophilic portions of the plastoglobules were much reduced in size. In the ripe red fruit, the plastoglobules had almost completely disappeared from the chromoplast stroma and only the crystals and fibrils remained.

Isolates from red fruit, prepared by negative staining, contained numerous fibrils and crystals, in addition to small portions of disrupted cell membranes. The fibrils had a diameter of about 20–30 nm, and their thickness varied somewhat along their length. Some still appeared to be attached to plastoglobules (large roundish structures, about 0.2 μm in diameter – Fig.4). The fibrils were straight or slightly bent and their length varied. The longest

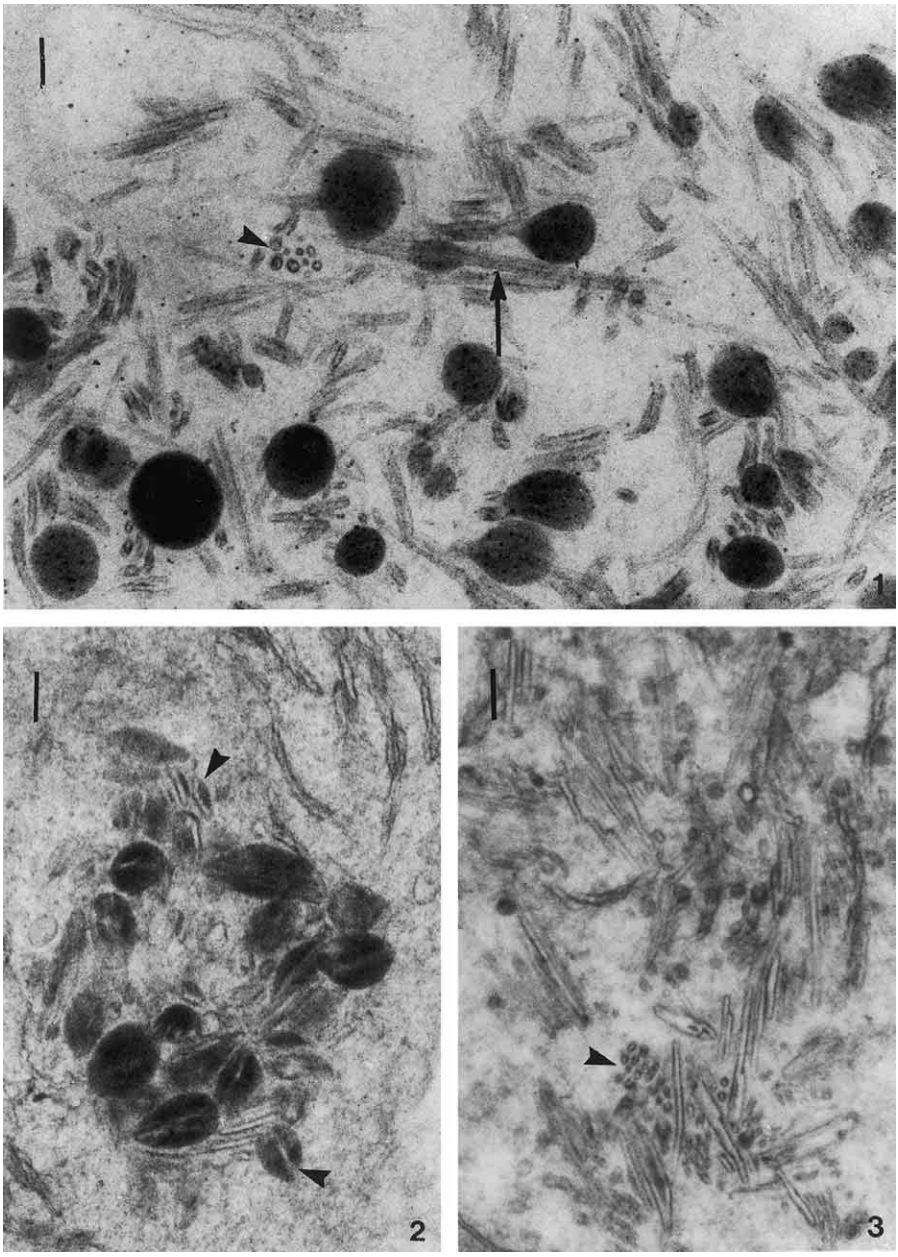

Fig. 1. Thin section through a chromoplast from an orange (half-ripe) fruit of *Solanum capsicastrum*. TCH-Ag-proteinate-staining. Fibrils are in contact with plastoglobules (arrow). Cross-sectioned fibrils are indicated (arrowhead). Bar = 0.1 m.

Fig. 2. Thin section through a chromoplast from an orange *S. capsicastrum* fruit. Some of the plastoglobules contain crystalloid inclusions (arrowheads). Bar = 0.1 m.

Fig. 3. Thin section through a chromoplast from a ripe red fruit with a group of crystals. Cross-sectioned fibrils are among the crystals (arrowhead). Bar = 0.1 m.

measured was about 5 μm, while most of them were much shorter, most likely because they were fractured during preparation.

The crystals, that were present on the same grid (Fig. 4), were very thin plates without any visible membrane (naked). The smallest ones were elongated oblique prisms with angles around 50°. Their lengths varied from about 150 nm till about 1 μm (Figs. 5, 6). Most crystals were larger (2–3 μm); they appeared to be aggregates of smaller subunits and their form was therefore often irregular (Fig. 4). The average thickness of the crystals was only about 1.5 nm, as calculated from the lengths of the shadows obtained after metal shadowing (Fig. 7).

Discussion

Plastoglobules are present in almost all types of plastids. Their content may vary according to the type of plastids (YTTERBERG et al. 2006). Accumulation of large quantities of pigments (carotenoids) is a particular characteristics of the globular type of chromoplasts. The pigments accumulate in plastoglobules and, in some plants, also in fibrils or crystals. In *Solanum* fruit, three types of pigment-containing substructures were present: plastoglobules and, in ripe fruit, crystals and fibrils. A similar accumulation of pigments was also found in the fruit of *Physalis alkekengi* (SIMPSON et al. 1978, LJUBEŠIĆ et al. 1999). Chromoplasts frequently contain two pigment-containing structures. Fibrils associated with plastoglobules were, for instance, observed in flowers of cucumber (PREBEG et al. 2006b) and *Impatiens noli tangere* (WRISCHER et al. 1999), and in pepper fruit (DERUÈRE et al. 1994, SALOPEK and LJUBEŠIĆ 1994). Pigment crystals developing inside the plastoglobules occur, for example, in the chromoplasts of *Liriodendron* flowers (HLOUŠEK-RADOJČIĆ and LJUBEŠIĆ 1985). Plastoglobules are not always necessary for the formation of fibrils. In some cases, such as the flowers of *Tropaeolum majus* and *Chelidonium majus* (FALK 1976, PREBEG et al. 2006a), they start to develop in contact either with the inner membrane of the chromoplast envelope or some other plastid membrane. In these plants, plastoglobules can appear later during chromoplast differentiation, in contact with the fibrils, as was observed in flowers of *Hypericum perforatum* (LJUBEŠIĆ et al. 1995). Finally, in a large number of plants, carotenoids are present only in chromoplast plastoglobules (SITTE et al. 1980, CAMARA et al. 1995). The diversity of pigment-containing structures in chromoplasts is therefore considerable. Patterns may depend on the type and the quantity of the carotenoids present in an individual tissue, but also on other components recently found in plastoglobules, among which a number of proteins with suspected enzymatic functions in carotenoid biosynthesis (YTTERBERG et al. 2006) deserve particular attention.

The two techniques applied in the study of the crystals and fibrils in chromoplasts of *S. capsicastrum* fruit afforded complementary information on their morphology and development. Analysis of thin-sectioned fixed material was more suitable for the visualisation of fibrillar structures. Following TCH-Ag-proteinate staining (WRISCHER et al. 2001), the distribution of the lipid layers (dark regions) in cross-sectioned fibrils confirmed the published model of localization of their main constituents (KNOTH et al. 1986, PREBEG et al. 2006b). On the other hand, the shapes of the crystals were more clearly visible in micrographs of negatively stained or metal-shadowed isolates than in sectioned material, where they can only be observed in transverse section.

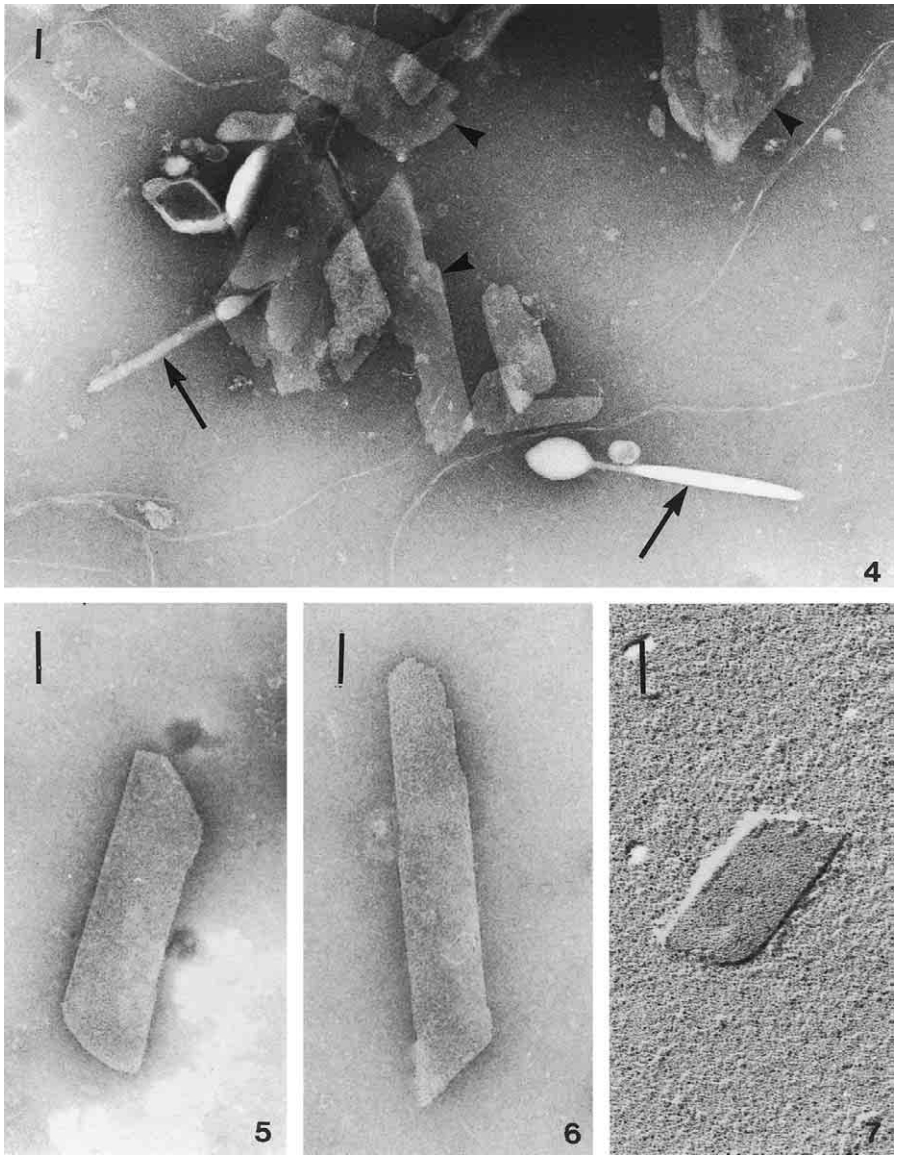

Fig. 4. Isolates from ripe red fruit negatively stained. Some fibrils are in contact with plastoglobules (arrows). Crystals are in groups (arrowheads). Bar = 0.1 m.

Figs. 5. and **6.** Crystals isolated from ripe red fruit negatively stained. Bar = 0.1 m.

Fig. 7. Crystal isolated from ripe red fruit shadowed with palladium. Bar = 0.1 m.

In thin-sectioned chromoplasts, the crystals and the core of the fibrils were only lightly stained indicating that they *in vivo* contained substances that were dissolved during tissue preparation (dehydration). We assume that these substances are carotenoids, which have already been identified in large quantities in *S. capsicastrum* fruit (SIMPSON et al. 1978).

Ripe fruit contain 51.3% β-carotene, 34% cryptoxanthin, and 14.5% lutein (LJUBEŠIĆ et al. 2001). Treatment of the fruit with norflurazon, an inhibitor od carotenoid biogenesis, also pointed to the presence of carotenoids in both crystals and fibrils. When flowers were treated with this herbicide, the subsequently developing fruit were pale-yellow, containing neither crystals nor fibrils in their chromoplasts, while the plastoglobules contained only lipids (LJUBEŠIĆ et al. 2001).

The form of the isolated crystals was similar to that of the carotene crystals found in carrot roots (KUHN 1970) and in petals of *Narcissus* flowers (HLOUŠEK-RADOJČIĆ and LJUBEŠIĆ 1988), and was also similar to synthetic β-carotene crystals (KUHN 1970). Characteristically, however, the crystals in *S. capsicastrum* develop inside the plastoglobules, in a lipophilic environment, while those of carrot and *Narcissus* are present in the hydrophilic thylakoid lumina (KUHN 1970). The paper of AUSTEN et al. (2006) confirmed that plastoglobules were formed at the halflipid bilayer of the thylakoids, i.e. in a lipophilic environment. It should be kept in mind that the true form of the crystals in *S. capsicastrum* can only be seen when isolated in aqueous medium. The thickness of the isolated platelets could only be calculated from the length of the shadows obtained after metal shadowing. Considering the pigment content of ripe fruit and the regular forms of the crystals, we assume that they contain β-carotene, while the other caroteinoids are localized in the fibrils. Separate isolation of crystals and fibrils would be required for unequivocal confirmation of their pigment content.

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