Ultrastructural Changes of Chloroplasts in Aging Tobacco Leaves

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ABSTRACT

In chloroplasts of mesophyll cells in senescing tobacco leaves, the first indication of aging is the formation of osmiophilic globuli. Concurrently, osmiophilic material accumulates in the cytoplasm adjacent to chloroplasts. Subsequently, starch grains and granal structure are lost. During advanced stages of disintegration, chloroplasts contain prominent osmiophilic globuli. Finally, even these plastoglobuli disappear, and all that remains in the cell are small, ill-defined membrane whorls.

Introduction

Chloroplast biogenesis has been extensively studied (reviewed: 17, 28). Chloroplast precursors contain vesicles, tubules, tubular aggregations (prolamellar bodies), and membrane-bounded inclusions, all of which are involved in synthesis of photosynthetic lamellae (reviewed: 31). Developing plastids also contain lipid droplets (plastoglobuli) which may function in lamellar formation (3, 34). The formation of large plastoglobuli is the most conspicuous and indicative change during chloroplast senescence (8). They are thought to be accumulation sites of membrane breakdown products during chloroplast degradation (5, 16, 18, 19, 20). This study examines plastid aging in tobacco leaves and describes final stages involving degradation of plastoglobuli and appearance of membrane whorls, stages which have not previously been described.

Materials and Methods

Tobacco plants, *Nicotiana tabacum* L. var. Beltsville C, were grown in the greenhouse. Leaves of varying age (partially expanded, fully expanded, yellow-green, and yellow leaves) were taken, beginning at 15 mm leaf primordia, from the 5th, 9th, 15th, and 18th nodes of plants approximately 50 cm in height.

Tissues from the mid-laminar region, between the midrib and leaf margin, were cut into 1 mm squares and fixed at room temperature in 3% glutaraldehyde in 0.05 M potassium phosphate buffer, pH 6.8. The tissue pieces were then rinsed in buffer and postfixed in 2% osmium tetroxide in buffer. All fixations were started at eight A.M. After dehydration in a graded series of ethanol followed by propylene oxide, specimens were embedded in araldite-epon (27). Thin sections were stained with uranyl acetate followed by lead citrate and examined with a Hitachi HU-11-B-2 electron microscope.

Results

In leaves expanded approximately two thirds maximum size, the chloroplasts were small (1-3 μ long) and elliptical in profile (Fig. 1).

The grana and intergrana lamellar systems were not elaborately developed. Starch grains were present in most chloroplasts.

Conspicuously larger chloroplasts (4-8 μ long) were found in fully expanded green leaves. At this stage, the thylakoidal system was highly developed. Large starch grains distorted lamellar structure and distended the external profile of chloroplasts (Fig. 2). Nearly all chloroplasts contained plastosomes (inset, Fig. 2), vacuole-like areas containing an electron-opaque matrix (see 2). Cytoplasmic pockets were occasionally present in these mature plastids; however, they were more common in proplastids (unpublished observations, see also 35).

Chloroplasts in yellow-green leaves had greatly enlarged plastoglobuli which disrupted lamellar structure and contained few starch grains (Fig. 3). Plastosomes were not observed in these chloroplasts. In the cytoplasm, adjacent to chloroplasts, were dense, osmiophilic deposits. Particularly large deposits occurred in the region between the chloroplast and cell wall.

In senescent, yellow leaves, plastids were small (1-2 μ long) and contained many, large plastoglobuli, no starch grains, and few lamellar remnants. Often, cytoplasmic membranes in concentric whorls were associated with these aged plastids (Figs. 4 and 5). Even in this late stage of degeneration, the plastid envelope, tonoplast, and plasma membrane remained intact (Fig. 4). Discontinuities were evident in the tonoplast at the same time that plastoglobuli appeared to be partially degraded (Fig. 5). In a later stage of chloroplast breakdown, the plastid envelope was discontinuous and the plastoglobuli were further degraded. Fully senescent cells were devoid of contents except for a membrane (probably plasma membrane) along the cell wall (Fig. 7) which was characterized by small membrane whorls and osmiophilic deposits (Fig. 6).

Discussion

In plant cells, the symptoms of aging are usually expressed first in the chloroplasts (8). The most conspicuous change is the deposition of osmiophilic substances in the plastid stroma. According to Arnott and Harris (2), tobacco chloroplasts contain two types of suborganelles, plastoglobuli and plastosomes, which act as lipid repositories.

During degeneration of tobacco chloroplasts, plastoglobuli increase in both number and size, an event reported in other studies (15, 22, 26). The closeness of plastoglobuli to thylakoids (5, 11) and their appearance as thylakoids break down (5, 7, 16) have led to the suggestion that plastoglobuli are repositories for lipids resulting from lamellar breakdown. Studies of the lipid composition of plastoglobuli substantiate this suggestion. Lichtenthaler (18, 19, 20) and Barr and Arntzen (4) have shown a correlation between lipoquinone content and number of plastoglobuli in aging plastids. Additionally, Lichtenthaler (19, 20) has shown that plastoglobuli contain carotenoids. Senescing chloroplasts resemble developing chromoplasts, where disappearance of chloroplast lamellae is accompanied by an accumulation of pigmented lipid droplets (21, 33).

Plastosomes, a term used by Arnott and Harris (2) to describe vesicles derived from unique membrane discs in the stroma of develop-

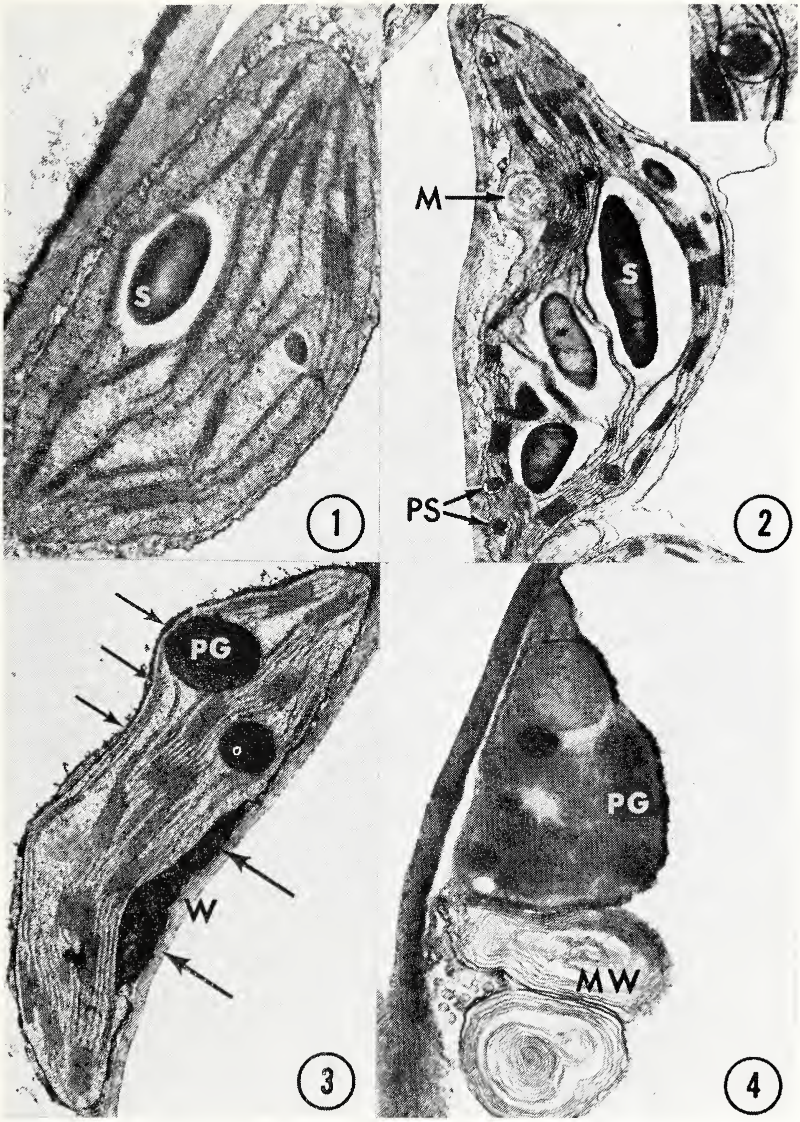
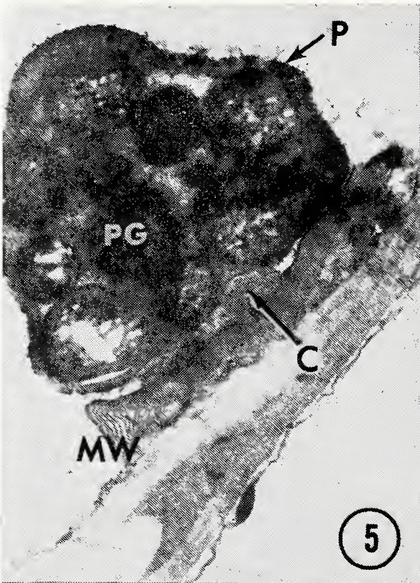


FIGURE 1. *Chloroplasts of partially expanded leaves have elliptical profiles. The lamellar system is not elaborately developed and small starch grains (S) are present in the stroma. X 36,000.*

FIGURE 2. *In fully expanded leaves, the chloroplast profile is distended by large starch grains (S). Cytoplasmic pockets in chloroplasts, though infrequent, may contain mitochondria (M). Plastosomes (PS) are common to chloroplasts at this stage of development. X 3,300. Inset shows detail of a plastosome. X 9,600.*

FIGURE 3. *Chloroplasts of yellow-green leaves contain large plastoglobuli (PG). Osmiophilic deposits occur in the cytoplasm adjacent to the plastid surface (small arrows) with massive deposits (large arrows) between the cell wall (W) and the plastid envelope. X 14,000.*

FIGURE 4. *Plastids of senescent, yellow tissue contain numerous plastoglobuli (PG). Cytoplasmic membrane whorls (MW) are common in cells at this stage of breakdown. X 18,500.*



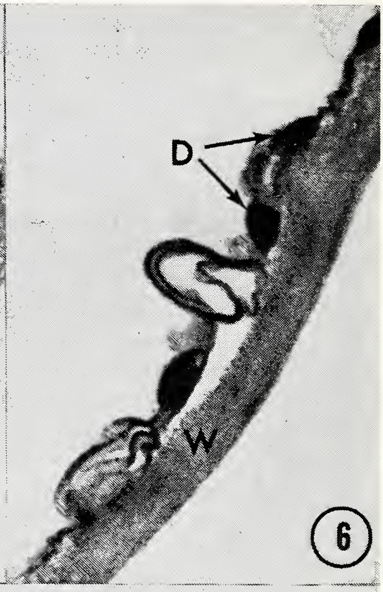


FIGURE 5. *Degradative changes in senescent, yellow tissue include loss of the tonoplast and breakdown of the plastoglobuli (PG). Between the plastid (P) and the membrane whorl (MW) is a remnant of the cytoplasm (C). X 20,000.*

FIGURE 6. *All that remains in senescent cells when plastids are completely degraded is an irregular membrane along the cell wall (W) which sometimes appears single, sometimes double, and sometimes whorled. Osmiophilic deposits (D) are typical of this membrane. X 66,000.*

FIGURE 7. *Cells of fully senescent tissue are devoid of contents except for a membrane (arrows) along the cell wall (W), probably residual plasma membrane. X 6,800.*

ing plastids, have only been described for tobacco. In agreement with Arnott and Harris (2), this study shows that plastosomes are an intermediate developmental stage preceding the appearance of plastoglobuli. Arnott and Harris (2) have shown a correlation between the appearance of plastosomes and a decrease in granal volume. Similar to plastoglobuli, plastosomes are partly lipid and may function in the isolation and/or release of metabolites during plastid development in tobacco (2).

In addition to lipid deposition within chloroplast suborganelles osmiophilic material accumulates in the cytoplasm adjacent to chloroplasts. Mittelheuser and Van Steveninck (24) reported formation of sperical lipid bodies in the cystoplasm of developing wheat leaves. These lipid bodies often distended the tonoplast and were occasionally observed between the chloroplast and plasma membrane. In contrast, cytoplasmic deposits in senescing tobacco leaves are amorphous masses adjacent to chloroplasts with massive deposits between the chloroplast and cell wall. Mlodzianowski and Kwinthiewicz (25) observed osmiophilic deposits adjacent to mitochondria, as well as chloroplasts, in detached, aging leaves of kohlrabi. They attributed deposition of osmiophilic material on the surface of these organelles to the action of lipophanerase.

Roux and McHale (32) considered degradation of the tonoplast and plasma membrane an early occurrence in senescence of detached tomato leaves. However, the plasma membrane and tonoplast are persistent in other senescing tissues (5, 7, 9, 12, 24). As shown in this study, degenerative changes accompanying cell senescence are well underway before the breakdown of these membranes. Since the loss of the tonoplast occurs at the same time as breakdown of the plastoglobuli and plastid envelope, it is reasonable to suggest that release of vacuolar contents may be responsible for final degradation of aged chloroplasts.

A characteristic of tobacco cells in terminal stages of senescence is the presence of myelin-like membrane whorls. Membrane whorls associated with the plasma membrane (13, 23, 30), endoplasmic reticulum (29, 30), vacuole (6, 10, 14), and thylakoids of plastids (1) have been found in developing tissues. In tobacco, myelin-like membrane whorls are a consistent feature of senescent leaf tissue, however, their origin is not known. They may be of vacuolar and/or plasma membrane origin since these membranes are disappearing at the same time that the myelin-like whorls appear in aging cells. Other studies have shown that membrane whorls can arise from the tonoplast (14) and the plasma membrane (13).

In general, the sequence of events described for chloroplast senescence in tobacco resembles that reported for other species (8). In addition, our study shows the final degradative changes of aged chloroplasts that were not reported previously. The generality of these terminal stages among plant species remains to be determined.

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