Light- and Electron-Microscope Studies on the Senescence of Chloroplasts in Elodea Leaf Cells

by Taiji IKEDA* and Rikizo UEDA*

Received July 4, 1964

While the development of submicroscopic structures in the chloroplast have been studied by several workers, little is known about the change in submicroscopic structures of degenerating chloroplast. Therefore, the present authors carried out studies using light and electron microscope on the structure change occurring in degenerating chloroplasts in leaf cells of Elodea densa.

Some of the cytochemical behaviors observed under the electron microscope are also described here.

Materials and Methods

The leaves of Elodea densa were used as material. They were detached from the stem and cultured in tap water at 27° in the dark for 1~15 days. Chloroplasts were observed by the use of light and electron microscope throughout the culture. Preparations available for electron microscope observation were made by the routine method as follows: Fresh leaves were fixed with 1% solution of osmium tetroxide (adjusted to pH 7.4 with acetate veronal buffer) for 1~2 hours, then dehydrated with a series of ethanol solutions of increasing concentration, and finally embedded in the monomer (n-butyl methacrylate: methyl methacrylate=6:4). Ultrathin sections were cut using JUM-5 ultramicrotome, and observed under JUM-5G electron microscope.

For the examination of pigmented granules in degenerating chloroplasts, some microchemical tests were applied together with densitometric analysis.

Results

Observations with light microscope: In general, mature chloroplasts in leaf cells of Elodea densa were green, and discoid in shape, being 6~7 μ in diameter. When plants were cultured in the dark at 27° the green color of the chloroplast changed gradually into pale yellow, and became smaller in size and irregular in shape, showing a symptom of degeneration. The senescent chloroplast of this type may be called as chromoplast, in which several yellowish granules can be observed at higher magnification of light microscope. As a rule, the size of these granules is larger than that of the normal grana. The process of chloroplast degeneration observed under the light microscope is shown in Table 1.

Observations with electron microscope: As shown in Fig. 1a, a cross section of mature chloroplast has a spindle-shaped appearance of about 6.5 μ in long diameter, and 0.9~1.3 μ in short one. The chloroplast is delimited by layered membranes of about 12 μ in thickness, the inner layers of which constitute a typical lamellar system. The grana lamellae and stroma lamellae were clearly recognized. The size of a single granum is about 0.6 μ in diameter, and 0.2 μ in thickness. The granum

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan.
Figs. 1, 2 and 3. 1a. A whole view of the fine structure of a mature chloroplast in leaf cell of Elodea densa. Low magnification: \( \times 19,000 \). 1b. The arrangement of lamella in grana. Higher magnification: \( \times 34,000 \). 2a, Chloroplast cultured in the dark for 3 days. \( \times 23,000 \). 2b, Senescent chloroplast after 5 day-culture. \( \times 22,000 \). 2c, Senescent chloroplast after 8 day-culture. \( \times 30,000 \). 2d, Senescent chloroplast after 11 day-culture. \( \times 35,000 \). 3a, Electron micrograph of senescent chloroplast. Control: The osmiophilic granules are noticeable. 3b, Treated with chloroform for 20 hours. The osmiophilic properties of granules in senescent chloroplast is obviously reduced.
are connected with each other by paired membranes protruding into the stroma (Fig. 1b).

The number of stroma lamellae is nearly equal to that of grana lamellae. A granum appeared to be a cylindrical pile composed of 6~8 disks; each disk being 0.12~0.15 μ in thickness, and 10 mμ in spacing. The end of grana lamellae which is connected with that of stroma lamellae is strongly osmiophilic.

Table 1. The process of chloroplast degeneration observed under the light microscope.

<table>
<thead>
<tr>
<th>Days in culture</th>
<th>Colour change of chloroplast</th>
<th>Diameter of chloroplast (μ)</th>
<th>Appearance of chloroplast</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Green</td>
<td>4.5-6.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Green</td>
<td>3.5-5.0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Yellowish green</td>
<td>1.5-3.0</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Yellow, far less green</td>
<td>0.5-2.0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Yellow</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

Some of the smaller osmiophilic granules of about 5.5 mμ in diameter are frequently found as freely dispersed among intergrana lamellae.

When the plant is cultured in the dark for 3~4 days no obvious destruction of lamellae system occurs in the whole appearance of the chloroplast. After 5 days, the shape of chloroplast changes, however, from discoid to sphere of about 2.4 μ in diameter. The lamellae in both grana and stroma become indistinguishable with each other, and irregular in arrangement. And, osmiophilic granules of 0.15~0.22 μ in diameter come into appearance in the chloroplast. After 8 days, the chloroplasts become smaller in size; the destruction of lamellar structure becomes more distinct and its size is ca. 350 mμ in diameter.

The changes in the structure of senescent chloroplasts in Elodea leaf, which was cultured in the dark, are shown in Fig. 2a~d.

Microchemical examination of the osmiophilic granules: It is quite likely that the yellowish granules observed in a senescent chloroplast under the light microscope are identical with osmiophilic granules seen under the electron microscope.

In order to examine whether these granules are made up of carotenoid or not, colour reactions and solubility tests were carried out by microchemical means under the light microscope."
Table 3. Sulfuric acid reaction on the materials pretreated with organic solvents (at room temperature).

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbon disulfide</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Benzene</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100% Acetone</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

-, Sulfuric acid reaction is negative. It is indicated that carotenoid is dissolved out from these granules.
+, Sulfuric acid reaction is positive.

The results are shown in Table 2 and 3, which have proved to be positive for carotenoid pigment.

As shown in Table 3, it is clear that the caroteneid in granules has been lost by treatment with chloroform, carbon disulfide, methanol, ethanol, and benzene. Therefore, it is likely that the treatment with these solvents is suitable for the detection of the carotenoid located in ultrastructure of the plastids.

From this reason, ultrathin sections were treated with organic solvents each for 20 hours, and observed electron-microscopically.

As shown in Fig. 3a and 3b and also in Fig. 4, the electron density in osmiophilic granules was reduced to a remarkable extent after such treatment. The experiment shows that the osmiophilic granules contain carotenoid pigment.

Discussion

The process of chloroplast degeneration was investigated by the use of light and electron microscope. It was disclosed that the degeneration of *Elodea* chloroplasts occurred in such a manner, decreasing the size destroying the lamellar structure, and increasing the number of osmiophilic granules. This was illustrated schematically in Fig. 5.

Strugger has shown that some of the small yellowish granules containing carotenoid pigment appeared in senescent chloroplasts of *Elodea canadensis*. Later, certain xanthophyll epoxide called “eloxanthin” has been obtained from *Elodea canadensis* by P. Karrer. According to Wettstein, the plastids in barley mutant, Xantha-3, increased in size, but no true lamellae have not developed in a typical fashion. Instead, a large number of globuli were formed in which carotenoid pigments were contained. Meanwhile, Frey-Wyssling reported that osmiophilic granules of 1,500Å in diameter were observed in plastids in the petal of *Ranunculus repens*. The structural organization of plastids in such cells is quite similar to that found in the chloroplasts of degenerated *Elodea* leaf observed by the present authors. The present experiment shows osmiophilic granules found in *Elodea* leaf may contain...
"eloxanthin" or some other carotenoids, which are probably combined with protein.

Minute osmiophilic granules are sometimes found also in normal mature chloroplast. An interesting question, whether the carotenoid-containing granules stated above are directly derived from these minute granules or they appear de novo in the process of chloroplast degeneration, remains still obscure.

A counterpart pertaining to the decay of lamellar system and the increment of osmiophilic granules indicates that the large osmiophilic granules are formed by

---

Fig. 4. Densitometric curves are showing the difference between lamella (L.) and osmiophilic granules (O. G.). In non-treated leaf, the density at L. is lower than that of O. G. and in leaf treated with chloroform, it is reversal. The result indicates that the carotenoid which concentrated in O. G. was lost.

---

Fig. 5. Schema showing the sequence of changes of structure in normal green chloroplast through etiolation. After 5 days the lamellar structure is destructed and diameter of chloroplast is shorter, and the granules become larger day after day.
condensation (Entmischung) of carotenoids liberated from the lamellar system. In Elodea cells, characteristic granules (S.G. in Fig. 2b) with a single envelope of 160 m,u=in diameter were frequently found in cytoplasm, but they were not removed even by treatment with organic solvents. Therefore, it seems that these granules are essentially different from those (O.G. in Fig. 2b) which have appeared in the course of degeneration process.

Summary

When the detached leaves of Elodea densa were cultured in tap water in the dark, chloroplasts were subjected progressive degeneration. After 5 days they became small in size and yellow in colour, having several yellowish particles.

Under electron microscope, the lamellar structure was degraded irregularly as the senescence proceeded, in which case the degradation of stroma lamellae seemed to occur earlier than that of grana lamellae. Moreover, it was found that some osmiophilic granules used to appear in the chloroplast. Those granules obviously correspond to the yellow particles observed under the light microscope. They reacted to produce a deep blue to blue-green colour by treatment with 75% H2SO4 and also showed a conspicuous iodine reaction. They are soluble in chloroform, methanol, carbon disulfide but insoluble in acetone and petroleum ether.

Therefore, it seems that some carotenoid pigments in lamellar structure were liberated and transferred into these granules. Probably, this may be identified as "eloxanthin", as previously called by Karrer.

References