J. Plant Biol. 38(3) : 275~280 (1995)

Ultrastructural Investigation on the Formation of Osmiophilic Globules in Ginseng Leaf Chloroplast by High Light

Ahn, Joung Sook, Chang-Seop Lee, Hoon Park[1] and Woo Kap Kim*
Department of Biology, Korea University, Seoul 136-701, Korea; and
[1]Korea Ginseng and Tobacco Research Institute, Taejon 302-345, Korea

The formation of osmiophilic globules related to the granal lysis has been investigated with a shade plant ginseng (*Panax ginseng* C. A. Meyer) exposed to full sunlight. The changes of chloroplast were examined as a function of time over 9 days under full sunlight exposure. The ultrastructure of ginseng leaf showed swelling of the granal thylakoid during an early stage of the light exposure. The thylakoid membrane faded and small electron-opaque dots were aggregated on the edges of the granal thylakoid membrane when the exposure time was increased over 1 day. Then, the shape of the grana changed into round. After the exposure over 3 days, there appeared many osmiophilic globules with multi-lamellated concentric structure. The globules at this stage were partly accumulated with osmiophilic substances. The outermost membrane of these multi-lamellated osmiophilic globules was attached to the stromal thylakoid membrane connecting to the deforming grana. The osmiophilic globules were elongated after 9 days. In this stage, the multi-lamellated structure was difficult to identify due to severe accumulation of osmiophilic substances. The number of the osmiophilic globules also increased along with the full sunlight exposure time. This observation leads us to believe that the multi-lamellated osmiophilic globules came from the deformation of grana.

Keywords : ginseng, grana, osmiophilic globules, thylakoid membrane, multi-lamellated osmiophilic globule

The root of ginseng is cultivated for medicinal purpose under shaded conditions. Various factors affect the growth of ginseng, of which light intensity is the most important factor and investigated very extensively (Park, 1980; Cheon, 1988). Exposure of a green plant to strong sunlight can result in a serious injury to photosynthetic apparatus. The exposure to strong light induces photoinhibition as a means of photoprotective responses. If shade plants are exposed to strong sunlight, excessive photoinhibition leads to photooxidation (Demig-Adams and Adams III, 1992).

Photooxidation of chlorophyll is an external symptom of intracellular damage occurring within the chloroplast. High light damage produces apparent changes on the ultrastructure and function of the chloroplast. Changes in the chloroplast ultrastructure are manifested by dilation of the thylakoid membranes, a progressive disruption of the thylakoid structure, and increases in the number of osmiophilic globules (plastoglobuli) within the chloroplast (Hernandez-Gil and Schaedle, 1973; Coloquhoun *et al.*, 1975; He *et al.*, 1994). Recently, Ahn *et al.* (1994) have reported that high light irradiance to ginseng leaf can result in a significant decrease in the CP-complexes particularly in LHCII and an increase in the number of osmiophilic globules according to degradation of the lamellar structure in the grana. Many other investigations have been performed on the ultrastructure of chloroplast in relation to leaf senescing. Their works seem to agree that the most striking feature is breakdown of the thylakoids, and the change is associated with an increase in the size

*Corresponding author: Fax +82-2-923-9522

and/or number of osmiophilic globules (Lichtenthaler, 1968; Dodge, 1970; Hurkman, 1979; Tuquet and Newman, 1980). The accumulated data indicate that the osmiophilic globules may represent accumulation of membrane break down, and their increase can be the most conspicuous indicator of damage in the chloroplast structure.

Although much attention has been paid previously to the osmiophilic globules, the formation process has not been clarified. In the present work, we have investigated break down of grana stacks, which is associated with the progressive formation of the osmiophilic globules. We have paid special attention to changes in the thylakoid membrane at initial stage of the high light influence.

MATERIALS AND METHODS

Materials

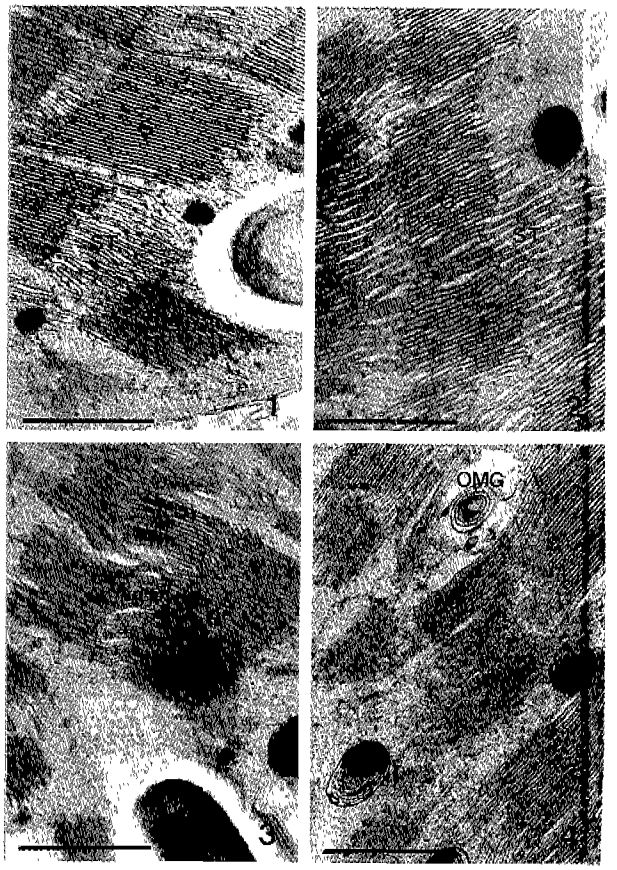
5-years old ginseng plants were grown under shaded conditions (5% sunlight, 5,000-7,000 Lux) for 2 months after emergence at the Korea Ginseng and Tobacco Research Institute. The plants were then exposed to full sunlight (100%, 120,000-150,000 Lux) for 9 days. Samples were collected 2, 4, and 8 h, 1, 3 and 9 days after the full sunlight exposure, respectively.

Ultrastructure

Leaves were dissected under fixative into 1 mm^2 pieces. The tissue pieces were fixed in 3% glutaraldehyde in a sodium phosphate buffer of pH 7.1 for 2 h, rinsed three times in same buffer and post fixed in 2% OsO_4 for 1.5 h. The tissue blocks were dehydrated in graded series of ethanol and embedded in Spurr's resin (Spurr, 1969). Silver sections were cut with an LKB-V ultramicrotome and collected on collodion coated copper grids, and then stained with uranyl acetate and lead citrate. Sections were examined with a transmission electron microscope (JEM 100 CX-I) operating at 80 kV.

RESULTS

Chloroplast ultrastructure of ginseng leaf from control (5% sunlight) showed characteristic of a typical shade plant. The chloroplasts possess typical well-developed granal and stromal thylakoids, which are generally oriented parallel to the long axis of the chloroplast. Granal thylakoids and the interconnecting stromal thylakoids are clearly visible (Fig. 1).



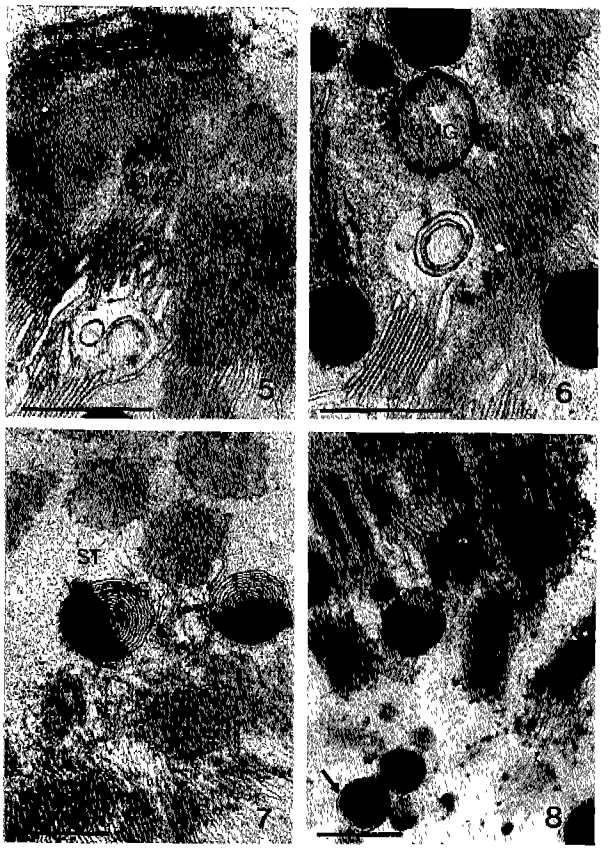
Figs. 1-4. Fig. 1. Electron micrograph for chloroplast in control leaf. Grana stacks are densely packed. Granal and stromal thylakoids are clearly visible. G, grana; ST, stromal thylakoid. Bar=0.5 μm. Fig. 2. Electron micrograph obtained after full sunlight exposure for 2 h. Thylakoid membranes begin to swell. Bar=0.5 μm. Fig. 3. Electron micrograph obtained after 4 h exposure. Thylakoid membranes are turning fade. Bar=0.5 μm. Fig. 4. Electron micrograph obtained after 8 h exposure. Small electron-opaque dots are aggregated on the edges of fading grana stacks. OMG, osmiophilic multi-lamellated globule. Bar=0.5 μm.

In contrast to control plants, leaf exposed to full sunlight for 2 h to 9 days showed a sequence of ultrastructural changes. When the leaf was exposed to full sunlight for 2 h, the membranes of granal and stromal thylakoids began to swell, although the internal membrane network was still intact (Fig. 2). With the exposure over 4 h, the lamellar system no

longer resembled the elaborate internal membrane system typical of mature chloroplast. Grana stacks were turning faded, having a disoriented lamellar system with the granal and stromal thylakoid becoming slightly swollen (Fig. 3). With the exposure over 8 h, changes in the ultrastructure were very conspicuous. Small electron-opaque dots were aggregated on the external surface of fading grana stacks, and the shape of grana stacks changed into round (Figs. 4, 5). With the exposure over 1 day, osmiophilic globules were enlarged to the size of adjacent grana. It was possible to find the early formation of some multi-lamellated osmiophilic globules with the accumulation of osmiophilic substances on the edge from the deforming grana (Fig. 6, arrow). With the exposure over 3 days, osmiophilic substances were partly accumulated inside of the multi-lamellated osmiophilic globules. The most important feature was that stromal thylakoid membrane joined the outermost membrane of the multi-lamellated osmiophilic globules to deforming grana (Fig. 7, arrows). The multi-lamellated osmiophilic globules were very similar in size with adjacent deforming grana stacks. When the exposure was increased over 9 days, the multi-lamellated osmiophilic globules were almost filled with osmiophilic substances. Much electron-opaque osmiophilic substances were densely accumulated on the edge of the fading grana stacks (Fig. 8). Although inside of the osmiophilic globules were almost filled with osmiophilic substances, the lamellar structure was still identified (Fig. 8, arrow).

The chloroplast from control had well developed grana stacks and few small osmiophilic globules ranging from 66 to 106 nm in size. The number of the osmiophilic globules increased gradually with the full sunlight exposure time (Figs. 9a, 9b, 9c, 9d). With the exposure over 9 days, osmiophilic multi-lamellated globules were not observed owing to full accumulation of electron-opaque substances. The size of the globules increased to 396-462 nm (Fig. 9d).

Fig. 10 shows variation in the number of osmiophilic globules per μm^2 in the chloroplast as the 100 % sunlight exposure time increased from 2 h to 9 days. The number of the osmiophilic globules showed a general increase with respect to the light exposure time. A deviation at 3 days from the general trend could be due to statistical fluctuation.



Figs. 5-8. Fig. 5. Electron micrograph obtained after 8 h exposure. Osmiophilic multi-lamellated globules begin to form with the accumulation of osmiophilic substances on the edge. Bar=0.5 μm. Fig. 6. Electron micrograph obtained after 1 day exposure. Osmiophilic multi-lamellated globules are developed, compared with Fig. 5. Bar=0.5 μm. Fig. 7. Electron micrograph obtained after the exposure for 3 days. Osmiophilic substances are partly accumulated inside of osmiophilic multi-lamellated globules. Stromal thylakoid membranes are connected with the outermost membranes of osmiophilic multi-lamellated globules (arrows). Bar=0.5 μm. Fig. 8. Electron micrograph obtained after the exposure for 9 days. The osmiophilic multi-lamellated globules are almost filled with osmiophilic substances, and much electron-opaque osmiophilic substances are densely accumulated on the edge of the fading grana stacks. Bar=0.5 μm.

DISCUSSION

High light stress is generally injurious to plants and, in particular, affects chloroplast. Photosynthetic activity decreases as a result of the stress. The structure of thylakoids, as well as the amount and ratio of chlorophylls, changes (Valanne, 1977). Osmiophilic globules begin to appear in chloroplast, and the

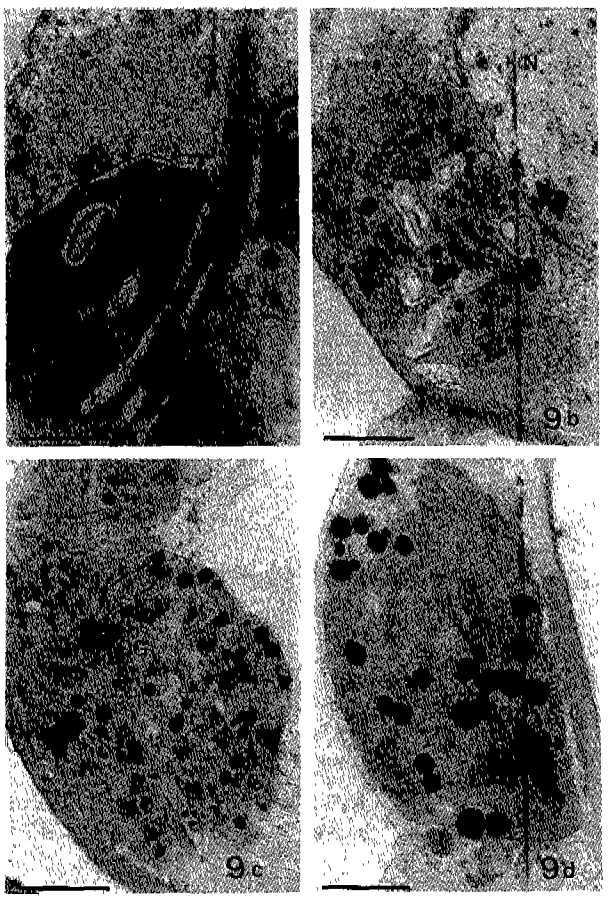


Fig. 9. Electron micrographs show that the number of osmiophilic globules increases with the light exposure time. OG, osmiophilic globule; N, nucleus; (a) control, (b) 1 day, (c) 3 days and (d) 9 days. Bar=1 μm.

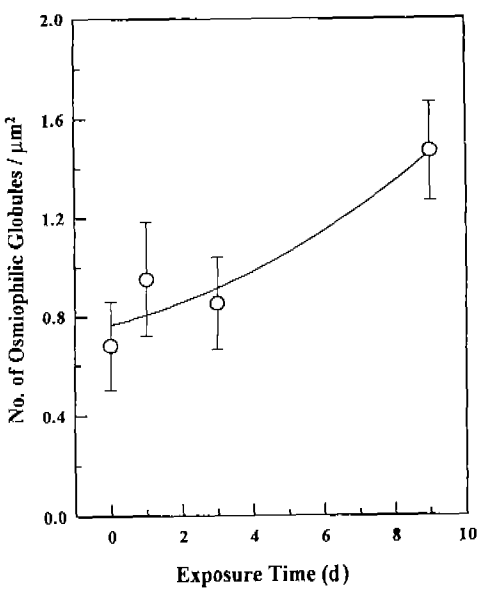


Fig. 10. The number of osmiophilic globules increases with the full sunlight exposure time.

formation has been connected with breaking of thylakoids (Aro and Valanne, 1979). Typical changes in the chloroplast ultrastructure during leaf senescence showed that the formation of osmiophilic globules (plastoglobuli) was the most common and conspicuous change (Lichtenthaler, 1968; Dodge, 1970; Hurkman, 1979; Tuquet and Newman, 1980). It is now well recognized that both the leaf senescence and high light bring about similar patterns of sequential changes in the chloroplast ultrastructure. The most remarkable morphological changes in chloroplast by high light are disappearance of thylakoids and increase in the number and size of osmiophilic globules (Aro and Valanne, 1979; Ahn *et al.*, 1994).

The osmiophilic globules are osmiophilic and spherical bodies observed in stroma of chloroplasts. Normal chloroplasts have few osmiophilic globules. However, the globules increase in number and size as thylakoids are degraded during leaf senescence (Harris and Arnot, 1973; Simpson *et al.*, 1978). It is therefore assumed that the osmiophilic globules have a function connected with thylakoid formation or breakdown (Tevini, 1977; Harris and Arnot, 1978). Osmiophilic globules may serve as pools for storage of thylakoid constituents especially of lipids. The complete composition of osmiophilic globule, however, is still unknown. Lichtenthaler (1968) has suggested that osmiophilic globules can be reservoirs for the excess lamellar lipids and especially for plastoquinones and esterified carotenoids which are not decomposed after breakdown of membranes in senescent chloroplast. Thi and Silva (1977) have believed that the osmiophilic globule is composed of proteins or lipids since the globule is formed from chloroplasts at the last stage of degradation and reacts with lipase, protease and phospholipase. Steinmüler and Tevini (1985) have suggested that glycolipids and proteins, the main constituents of thylakoids, do not play an important role in osmiophilic globule metabolism, are extensively degraded during senescence, but not stored within osmiophilic globule. However, carotenoid esters, prenyl quinones and fatty acids are deposited into osmiophilic globule. Ahn (1994) has reported that LHCII, the main thylakoid membrane protein, is not distributed in osmiophilic globules, using immunogold labeling of LHCII in chloroplast. These results indicate that the constituents of osmiophilic globules are lipids rather than proteins.

There are two groups of osmiophilic globules in chloroplast. The osmiophilic globules are observed throughout early development to the mature stage of chloroplast. The size of globules from control is 66-106 nm. However, the size has increased to 393-462 nm after sun light exposure in the present investigation. The difference of size indicates that the osmiophilic globules with a multi-lamellated structure forming with high light irradiance have a different origin, compared with the osmiophilic globules from control.

The osmiophilic globule with a multi-lamellated structure has been observed first in the present work. In the chloroplast of control, one granum is connected to another by stromal thylakoids. In photodamaged chloroplast, on the contrary, a deforming granum is connected to the outermost membrane of multi-lamellated osmiophilic globule by stromal thylakoids (Fig. 7, arrows). This difference leads us to suggest that the osmiophilic multi-lamellated globule comes from the deforming grana. In addition, the grana degradation progresses as the ginseng leaf is exposed to sunlight. The number and size of the osmiophilic globules increase along with the granal degradation (Figs. 9, 10). The accumulation of osmiophilic substances also increases with the sunlight exposure (Figs. 5-8). Eventually, at the final stage of the granal destruction, the lamellar structure is difficult to identify (Fig. 8). From all these results, we believe that the osmiophilic globules are formed from degraded grana stacks, and the osmiophilic substances in the globules come from the breakdown of the granal lipids.

LITERATURE CITED

Ahn, J.S. 1994. Changes of chloroplast ultrastructure and thylakoid membrane proteins by light intensity in ginseng (*Panax ginseng* C. A. Meyer) leaves. Ph. D. Thesis. Korea Univ., Seoul.

Ahn, J.S., B.G. Cho, H. Park and W.K. Kim. 1994. Changes in chloroplast ultrastructure and thylakoid membrane proteins by high light in ginseng leaves. *J. Plant Biol.* **37**: 285-292.

Aro, E.-M. and N. Valanne. 1979. Effect of continuous light on CO_2 fixation and chloroplast structure of the mosses *Pleurozium schreberi* and *Ceratodon purpureus*. *Physiol. Plant.* **45**: 460-466.

Cheon, S.K. 1989. Effects of light intensity and quality of Korean ginseng. Ph. D. Thesis. Kyungbook National Univ., Taegue.

Coloquhoun, A.J., J.R. Hillman, C. Grewe and B.G. Bowes. 1975. An ultrastructural study of the effects of abscisic acid in senescences of leaves of radish. *Protoplasma* **84**: 205-221.

Demig-Adams, B. and W.W. Adams III. 1992. Photoprotection and other responses of plants to high light stress. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **43**: 599-626.

Dodge, J.D. 1970. Changes in chloroplast fine structure during autumnal senescence of *Betula* leaves. *Ann. Bot.* **34**: 817-824.

Harris, J.B. and H.J. Arnot. 1973. Effects of senescence on chloroplasts of the tobacco leaf. *Tissue Cell* **5**: 527-544.

He, J., L.-K. Huang and M.I. Whitecross. 1994. Chloroplast ultrastructure changes in *Pisum sativum* associated with supplementary ultraviolet (UB-B) radiation. *Plant cell and Environment* **17**: 771-775.

Hernandez-Gil, R. and M. Schaedle. 1973. Functional and structural changes in senescing *Populus deltoides* chloroplasts. *Plant Physiol.* **51**: 245-249.

Hurkman, W.J. 1979. Ultrastructural changes of chloroplasts in attached and detached, aging primary wheat leaves. *Amer. J. Bot.* **66**: 64-70.

Lichtenthaler, H. K. 1968. Plastoglobules and fine structure of Plastids. *Endeavour* **27**: 144-149.

Park, H. 1980. Physiological response of *Panax ginseng* to light. *Proc. 3rd Int. Ginseng Sym.*, Korea Ginseng Res. Inst. Seoul, 151-170.

Simpson, D.J., M.R. Baqar and T.H. Lee. 1978. Chromoplast ultrastructure in fruits of *Solanum pseudocapsicum* and fruits and sepals of *Physalis alkekengi*. *Aust. J. Bot.* **26**: 793-806.

Spurr, A.P. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**: 31-43.

Steinmüler, D. and M. Tevini. 1985. Composition and function of plastoglobuli. *Planta* **163**: 201-207.

Tevini, M. 1977. Light, function and lipids during plastid development. *In*, Lipid and lipid polymers in higher plants, M. Tevini and H.K. Lichtenthaler (eds.), Springer Berlin Heidelberg New York, pp. 121-145.

Thi, A.T.P. and J.G. Da Silva. 1977. Sur la nature biochemique des corps intrachloroplastiques chez le contonnier. compt. rend. *Acad. Scie. (Paris) Ser. D.* **284**: 2107-2109.

Tuquet, C. and David W. Newman. 1980. Aging and regreening in soybean cotyledons. I. Ultrastructural changes in plastids and plastoglobuli. *Cytobios* **29**: 43-59.

Valanne, N. 1977. Effect of continuous light on CO_2 fixation, chlorophyll content, growth and chloroplast structure in *Ceratodon purpureus*. *Z. Pflanzenphysiol.* **81**: 347-357.

(Received July 4, 1995)

强光에 의한 人蔘잎 葉綠體의 好 오스뮴性 小球體의 形成에 관한 微細構造的 硏究

安 丁 淑 · 李 昌 燮 · 朴 薰[1] · 金 宇 甲*
高麗大學校 理科大學 生物學科, [1]韓國人蔘煙草硏究院

적 요

인삼(*Panax ginseng* C. A. Meyer) 잎을 대상으로 9일간 광노출 후 변화되는 엽록체 내의 그라나 붕괴에 따른 호 오스뮴성 소구체의 형성 과정을 연구하였다. 광노출 초기의 그라나 틸라코이드는 팽창 현상을 보였다. 광노출 1일 후에는 틸라코이드 막이 흐려지고 그라나 틸라코이드 말단 주변부에 전자 밀도가 높은 과립들이 출현하였으며 흐려진 그라나는 전체적으로 둥근 모양으로 변형되었다. 광노출 3일 후에는 동심원 층상 구조의 호 오스뮴성 다층 막성 소구체들이 많이 관찰되었고 이 층상 구조는 전자 밀도가 높는 물질들로 일부 축적되어 있었다. 호 오스뮴성 다층 막성 소구체의 최외각 막이 변형된 그라나와 연결된 스트로마 틸라코이드와 연결되어 있는 것이 관찰되었다. 광노출 9일에는 호 오스뮴성 소구체의 크기가 크게 신장하였으며, 호 오스뮴성 물질들로 충만하여 동심원의 층상 구조는 관찰하기 힘들었다. 이러한 결과에 의해 호 오스뮴성 다층 막성 소구체는 그라나의 붕괴에 의하여 형성된다고 사료된다.

주요어 : 인삼, 그라나, 호 오스뮴성 소구체, 틸라코이드 막, 호오스뮴성 다층 막성 소구체

*교신저자 : Fax (02) 923-9522