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Chloroplast Microtubules in Young Leaves of *Sedum rotundifolium*

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Well-defined tubular inclusions were detected in mesophyll chloroplasts of young *Sedum rotundifolium* leaves. The size and distinctly uniform arrangement of tubular inclusions were the most noticeable features. The chloroplast usually contained a large inclusion, sometimes extending almost as long as the chloroplast. Such inclusions were built up from microtubules exhibiting aggregates of either large plate-like or paracrystalline structures depending on the section angles. These inclusions were often quite huge, measuring as much as 7.1 μm in length and 2.6 μm in width. The diameter of the microtubule was approximately 25 nm. The microtubule aggregates were non-membrane bounded structures enclosed partly by the thylakoids. The microtubules in the aggregate were all displaying the same definite orientation. Cross-sectional views clearly demonstrated the characteristic hexagonal arrangement within paracrystalline structures. Longitudinal sections of the chloroplast microtubules showed that they were in perpendicular orientation to the chloroplast envelope. They were not connected to these membranes in any case. In general, one microtubular aggregate was seen in each chloroplast section. The role of tubular inclusions in the chloroplasts related to the photosynthetic mode was discussed.

Keywords: chloroplast microtubules, *Sedum rotundifolium*, young leaves, CAM

Microtubules in the chloroplasts were earlier reported in algal species (See Santos and Salema, 1981). They were also described in various plastids of higher plant species. Proplastids of specialized tissues in *Acacia* (Rickson, 1975) and chromoplasts of reproductive tissues such as calyx (Sitte, 1974), petals (Ljubesic, 1979), and fruits (Spurr and Harris, 1968) have revealed such tubular structures. In angiosperms, mature chloroplasts with microtubule structures are not commonly known among C-3 plants. Only a few species of C-4 succulents were recorded to have microtubules in their chloroplasts (Kim and Fisher, 1990), whereas they have been rather frequently reported in plants performing Crassulacean acid metabolism (CAM) mode of photosynthesis. Microtubules in chloroplasts of a higher plant were first discovered in mesophyll cells of *Sedum telephium*, CAM species, where they formed huge paracrystalline inclusions (Brandao and Salema, 1974). Hence, these chloroplast tubular structures have been described in various studies dealing with CAM species of *Crassula* (Kapil *et al.*, 1975), *Echinomastus* (Rivera and Arnott, 1982), *Kalanchoe* (Kapil *et al.*, 1975; Santos and Salema, 1981), *Lithops* (Santos and Salema, 1981), and *Sedum* (Brandao and Salema, 1978; Salema and Brandao, 1978; Santos and Salema, 1983). Since many CAM plants exhibited microtubules in their chloroplasts, a possible relationship between microtubules and the CAM pathway has been strongly speculated (Santos and Salema, 1983).

During a series of electron microscopic studies on the foliar structural differentiation of several succulent species, well-defined tubular structures were detected in almost all of the mesophyll chloroplasts of young *Sedum rotundifolium* leaves. No studies have yet been performed with respect to the ultrastructure of this species. Thus, the current report is a description of such tubular inclusions found in their mesophyll chloroplasts. The study also attempted to compare the amount of tubular inclusions and starch content in the chloroplasts by surveying materials fixed both in the morning and in the afternoon. However, the present study mainly focused on tubular inclusions in young leaves of *Sedum rotundifolium* in relation to the chloroplast ultrastructure of the meso-

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phyll cells.

MATERIALS AND METHODS

Young leaves, ca. 10 mm in length, were sampled in the laboratory from the several plants of *Sedum rotundifolium* collected from Mt. Juwang, Kyungpook in September 1996. Ca. 1-2 mm pieces were dissected and fixed with 3% glutaraldehyde for 3 hrs at room temperature and followed by 2% osmium tetroxide. They were buffered in each case with 0.1 M potassium phosphate at pH 6.8-7.4 as described previously (Kim *et al.*, 1995). Fixed materials were dehydrated through a graded acetone series and the specimen were embedded in Spurr's resin (Spurr, 1969). They were polymerized for 48 hrs at 68°C. For comparison of the tubular inclusion and starch content in the chloroplasts, samples were collected at both 08:00-08:30 and 14:00-14:30. Semi-thick sections, ca. 0.5-1 μm, and 60-90 nm ultrathin sections were made with a Reichert 7000 ultramicrotome using glass and diamond knives. Semi-thick sections were stained with toludine blue in 5% Na tetraborate and examined with a Zeiss photomicroscope. Ultrathin sections were stained with 2% aqueous uranyl acetate and lead citrate for 45 min each and examined with Philips EM201 operated at 60 kV.

RESULTS

The mesophyll in young leaves of *Sedum rotundifolium* consisted of water-storing cells containing a somewhat large central vacuole bounded by a thin, peripheral layer of cytoplasm (Fig. 1). The presence of the chloroplasts and their mesophyll index (Kluge and Ting, 1978; De Santo and Bartoli, 1996) in these mesophyll cells are characteristic of CAM plants. Their mesophyll cells present no unusual or distinctive features in the light microscopy, while tubular inclusion bodies in the chloroplasts were remarkably distinct by electron microscopy. The chloroplasts included well-defined tubular inclusion, abundant starch grains, little plastoglobuli, and rudimentary to moderately developed thylakoids. These chloroplasts were highly variable structure and their shape was often influenced by the presence and shape of the tubular inclusions and starch grains (Fig. 2).

One of the most interesting features noticed in this study was the size and distinct arrangement of tubular inclusion in the chloroplasts. The chloroplasts usually had a large inclusion, extending through the stroma, sometimes from one side to the other (Fig. 3). These were almost as long as the chloroplast and thylakoids partly enclosed the structures. The inclusions were non-membrane bounded large structures, often measuring as much as 7.1 μm in length and 2.6 μm in width. The diameter of tubular elements were found to be approximately 25 nm and appeared to be surrounded by several subunits, although details of subunits in their wall was not examined in this study. Frequently paracrystalline inclusions as a huge aggregate consisting of several thousands, sometimes more than 10,000 elements, were encountered. These elements were not in direct contact with the neighboring ones. Arrays of tubular structures occupied a large volume of the chloroplasts, roughly up to 15-20% in some chloroplasts.

Fig. 1-3. Mesophyll cells showing thin peripheral layer of cytoplasm and large central vacuoles (V). IS=Intercellular space. Scale=10 μm. 2. Longitudinal section of chloroplasts containing paralleled tubular aggregates (arrow heads). CW=Cell wall, mb=Microbody, M=Mitochondria, S=Starch grain. Scale=0.7 μm. 3. A tubular inclusion (arrow heads) extending from one end to almost the other side of the chloroplast. Scale=1 μm.

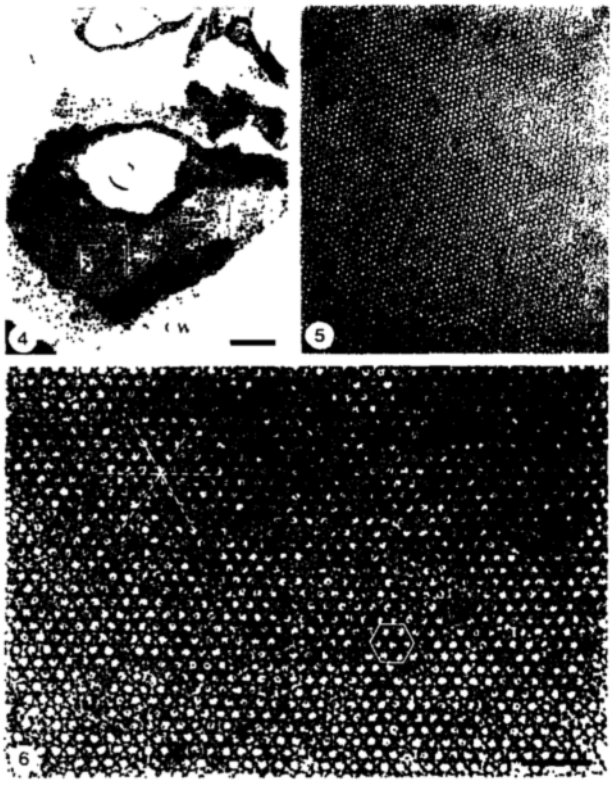

Fig. 4-6. Cross-sectioned chloroplast with a paracrystalline aggregate. Scale=1 μm. 5. The enlargement of [illegible] area from Fig. 4, demonstrating well-defined paracrystalline structure. Scale=0.2 μm. 6. Higher magnification of Fig. 5 clearly showing hexagonal arrangement of the microtubules (See dotted lines and a hexagon). Missing tubular elements are indicated by arrows. Scale = 0.1 μm.

Such inclusions were built up from microtubules, aggregated in large paracrystalline structures in cross sections (Figs. 4-6) or paralleled plate-like structures in longitudinal sections (Figs. 2-3). Missing elements were also observed within the structure. The microtubules in the aggregate were set up in such a way that each one was surrounded by six others (Fig. 6). Cross-sectional views clearly demonstrated the characteristic hexagonal arragement. In the aggregate, even though there were several places where a microtubule was missing, the hexagonal arrangement of the microtubules was still easily seen. In many cases, these microtubules appeared to be attached to the inner chloroplast envelope at one side and to the thylakoids at the other side (Figs. 2-4). Longitudinal sections of the chloroplast microtubules showed that they were in perpendicular orientation to the chloroplast envelope, but they were not connected to these membranes in any case. Microtubules in the large aggregate were all displaying the same definite orientation. They appeared to be compressed against each other by the thylakoids located around them. In general, one microtubular aggregate was seen in each chloroplast section. Rarely more than one tubular inclusion was encountered in this *Sedum* species. Features described above were based on the materials fixed in the afternoon. However, no considerable variation of tubular inclusion and starch content were detected between samples fixed in the morning and in the afternoon.

DISCUSSION

Tubular inclusions in plastids have been described in various studies dealing with CAM plants and had been named as stromacenter in an earlier work (Lee and Thompson, 1973). Later, these homogenously uniformed tubular structures have been considered as microtubules based on the nature of preservation by certain fixatives, disassembly and reassembly of organization by the special treatments, pronase sensitivity, average dimensions, presence of subunits in their wall, and microtubular behavior (Salema and Brandao, 1978). The present study also agrees with the views of Salema and Brandao (1978) considering the tubular inclusions in the chloroplasts as microtubules by their structural attributes. The number of microtubules in the stroma of plastids from various CAM species of higher plants varied greatly from one species to another. Some had huge aggregates of microtubules, whereas others had smaller groups. Huge aggregates were observed in the *Sedum rotundifolium*. In some cases, the electron micrographs of an inclusion found in chloroplasts of *Kalanchoe, Umbilicus*, and other *Sedum* species published by Thompson *et al.* (1977) and Santos and Salema (1981, 1983) were remarkablely similar to the chloroplast inclusions examined in *S. rotundifolium*. As in *Sedum spectabilis* where only one aggregate was observed in each chloroplast section (Santos and Salema, 1981), the aggregate of *S. rotundifolium* also mostly exhibited one inclusion showing either a large plate or paracrystalline structure depending on the section angles. According to Brandao and Salema (1974), the diameter of microtubules were found to be 250 Å, the wall measuring 50 Å in thickness, and the central light core 150 Å with a centere-to-center 300 Å spacing. The distance from one side of the hexagon to the other side is recorded to be about 8 nm apart. The diameter and size of the microtubule examined in *S. rotundifolium* were within the range revealed in other

CAM species. Their uniformity in appearance in all planes of section indicates that they were plates rather than other structures of rods and fibrils (Thomson and Journett, 1970).

Microtubules in plastids may have a variety of functions depending on the plant and tissue. The structure of the microtubule containing plastids may be related to their physiology (Rivera and Arnott, 1982; Gunning and Steer, 1996). The role of plastid tubular inclusion in a plastid division and development has been proposed (Vaughan and Wilson, 1981), but the most attractive hypothesis for the role of this structure is that the inclusion body stores enzymes necessary for the operation of CAM (Thompson *et al.*, 1977). Studies showed that microtubules formed in CAM induced plants through NaCl treatment (Salema and Brandao, 1978) or disassembled by cold treatments (Brandao and Salema, 1974). These results pointed to a possible link between the tubular inclusion of the plastids and CAM mode of photosynthesis (Salema and Brandao, 1978). Accumulated data on the tubular aggregates of some chloroplasts seem to indicate that some kind of relationship may exist between them and the CAM-type photosynthesis. The structure-function relationship investigated by Santos and Salema (1983) by studying the behavior of chloroplast tubules over 24 hr period indicated dramatic daily variation of the amount of microtubules. The variation of the amount of tubular inclusion in the chloroplasts of *Sedum telephium* was found to build up during the day followed by rapid disaggregation during the night. Up to 21% of the plastid volume occupied by the tubules were reported in this species (Santos and Salema, 1983). A study of various CAM plants including species of *Umbilicus, Kalanchoe, Sedum, Lithops* indicated that the protein from which chloroplast microtubules are built up is very likely an enzyme linked to the primary CO_2 fixation, possibly malic dehydrogenase (Santos and Salema, 1981). Thompson *et al.* (1977) also considered the inclusion as made up from an enzyme of the CAM scheme and further the possibility of the protenaceous nature of the tubular form as an enzyme of the PEP-cycle was suggested (Santos and Salema, 1983). It is, however, interesting to note that microtubules have been found so far only in chloroplasts of species belonging to the CAM NADP-malic enzyme rich subgroup (Santos and Salema, 1981). It could be an enzyme by the fact that microtubule is protein in nature (Brandao and Salema, 1974; Thompson *et al.*, 1977; Santos and Salema, 1983). Some specific structural proteins are known to be the essential plastid constituents (Emter *et al.*, 1990; Fosket, 1994). It seems more plausible that certain CAM species have microtubules in their chloroplasts because their cellular and organelle characteristics set the right conditions for polymerization and depolymerization of whatever protein is involved (Santos and Salema, 1983). Further microtubules as normal plastid constituents that are not distinguished in material due to the endogenous phenolic compounds, obscuring the tubule structures has been proposed by Vaughn and Wilson (1981). The consistency of these microtubules associated with the thylakoids and the inner envelope, and with structural arrangements in the plastids strongly indicate that these microtubules may have a dynamic and structural role in the chloroplasts (Vaughn and Wilson, 1981).

It must be, however, stressed that the presence of microtubules in the chloroplasts is probably due to the cellular properties that are able to establish the right conditions for the assembly of microtubules with proteins, yet unknown, involved in the process as suggested by Santos and Salema (1983). Necessity to deepen the analysis of such process should be carried out before a definite conclusion can be drawn. It also needs to be investigated whether daily fluctuation of the tubular inclusions as revealed in *S. telephium* takes place in mesophyll chloroplasts of *S. rotundifolium* by analyzing tissues with hourly intervals. Studies are underway to see if there is any correlation between leaf age and the differences found in the amount of tubular inclusions in *S. rotundifolium*.

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LITERATURE CITED

Brandao, I. and R. Salema. 1974. Microtubules in chloroplasts of a higher plant (*Sedum* species). *J. Submicr. Cytol.* **6**: 381-390.

De Santo, A.V. and G. Bartoli. 1996. Crassulacean metabolism in leaves and stems of *Cissus quadrangularis*. In: Crassulacean Acid Metabolism: Biochemistry, ecophysiology and evolution. (eds.) Winter, K. and J.A.C. Smith. Springer, Berlin. pp. 216-229.

Emter, O., H. Falk and P. Sitte. 1990. Specific carotenoids and proteins as prerequisites for chromoplast tubule formation. *Protoplasma* **157**: 128-135.

Fosket, D.E. 1994. Plant Growth and Development. Academic Press. New York. pp. 210-218.

Gunning, B.E.S. and M.W. Steer. 1996. Plant Cell Biology: Structure and Function. Jones and Bartlett Publishers, Boston. pp. 1-60.

Kapil, R.N., T.D. Pugh and E.H. Newcomb. 1975. Microbodies and an anomalous "microcylinder" in the ultrastructure of plants with Crassulacean acid metabolism. *Planta* **124**: 231-244.

Kim, I.S. and D.G. Fisher. 1990. Structural aspects of the leaves of seven species of Portulaca growing in Hawaii. *Can. J. Bot.* **68**: 1803-1811.

Kim, I.S., J.H. Pak, S.B. Seo and S.D. Song. 1995. Foliar ultrastructure of Korean *Orostachys* species. *Kor. J. Electron Micros.* **25**: 457-463.

Kluge, M. and I.P. Ting. 1978. Crassulacean Acid Metabolism. Springer-Verlag, Berlin. pp. 5-44.

Lee, R.E. and A. Thompson. 1973. The stromacentre of plastids of *Kalanchoe pinnata* Persoon. *J. Ultrastruct. Res.* **42**: 451-456.

Ljubesic, N. 1979. Chromoplasts in the petals of *Liriodendron tulipifera* L. *Zeit. Pflanzenphysiol.* **91**: 49-52.

Rickson, F.R. 1975. The ultrastructure of *Acasia cornigera* L. Beltian body tissue. *Amer. J. Bot.* **62**: 913-922.

Rivera, E.R. and H.J. Arnott. 1982. Tubular structures in the plastids of *Echinomastus intertextus* Brit. & Rose (Cactaceae). *New Phytol.* **90**: 551-561.

Salema, R. and I. Brandao. 1978. Development of microtubules in chloroplasts of two halophytes forced to follow Crassulacean acid metabolism. *J. Ultrastruct. Res.* **62**: 132-136.

Santos, I. and R. Salema. 1981. Chloroplast microtubules in some CAM plants. *Bol. Soc. Brot. Ser.* **53**: 1115-1122.

Santos, I. and R. Salema. 1983. Stereological study of the variation of chloroplast tubules and volume in the CAM plant *Sedum telephium*. *Zeit. Pflanzenphysiol.* **113**: 29-37.

Sitte, P. 1974. Plastid metamorphosis and chromoplasts in *Chrysosplenium*. Zeit. Pflanzenphysiol. **73**: 243-265.

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

Spurr, A.R. and W.M. Harris. 1968. Ultrastructure of chloroplasts and chromoplasts in *Capsicum annum* L. Thylakoid membrane changes during fruit ripening. *Amer. J. Bot.* **55**: 1210-1224.

Thomson, W.W., and R. de Journett. 1970. Observations on inclusion bodies in subsidiary cells of *Opuntia* species. *J. Ultrastruct. Res.* **31**: 439-443.

Thompson, A., J. Vogel and R.E. Lee. 1977. Carbon dioxide uptake in relation to a plastid inclusion body in the succulent *Kalanchoe pinnata* Persoon. *J. Expt. Bot.* **28**: 1037-1041.

Vaughn, K.C. and K.G. Wilson. 1981. Improved visualization of plastid fine structure: plastid microtubules. *Protoplasma* **108**: 21-27.

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