Electron Microscopic Observation of the Balloon-Formation of Isolated Spinach Chloroplasts

It has been found that when the isolated chloroplasts are suspended in distilled water or hypotonic solutions, they become balloon-like forms as a result of the extension of the membranes[1–5]. It was previously reported that the balloon-formation of chloroplasts isolated from spinach and *Nitella* was observed in 2 different types with photomicroscope[5]. MERCER et al.[1] found with electron microscope that the balloon results from the swelling of limiting membrane in *Nitella* chloroplasts, but SPENCER and WILDMAN[3] considered, on the basis of photomicroscope, that in spinach the formation results from the extension of stroma lamellae. However, many investigations[2–5] of the balloon-formation were made almost exclusively with photomicroscope, except for the report described by MERCER et al.[1]. There are also electron microscopic studies on the structural changes without the balloon-formation of chloroplasts in hypotonic solutions[6–8].

In the present experiments, the detailed structure of the balloon of spinach chloroplasts was studied and the formation of the balloon was discussed.

Spinach chloroplasts were isolated in 20 m*M* *Tris*-HCl buffer (pH 7.5) containing 0.4 *M* sucrose or 0.3 *M* NaCl, as previously described[9]. The balloons were formed by suspending the chloroplasts in distilled water or a solution of 0.2 m*M* $MgCl_2$. The balloons were observed with JEM-6A electron microscope after the samples were prepared using the method of IZAWA and GOOD[10].

It was photomicroscopically observed that the balloons were spherical forms with 12 μ (range: 8–14 μ) diameter as in other observations[1–5]. No significant difference was found between the number of the balloons formed from salt chloroplasts and from sugar ones (the number was counted with haematocytometer). Thus, the balloons were formed from the chloroplasts lacking limiting membranes as well as intact chloroplasts. It therefore seemed that the balloon of spinach chloroplasts is not formed from limiting membrane as described by MERCER et al.[1] but from stroma lamellae as described by SPENCER and WILDMAN[3].

The balloons were further observed with electron microscope. The balloons in our sections were not observed in a spherical form but depressed (Figure 1) or flattened in form (Figure 3), probably because of the deformation during fixation, dehydration and embedding. In many cases, a part of the balloon envelope had the various structures, such as grana discs, tangle, or vesicles (Figures 2–4), while the balloon without such structure was observed only rarely (Figure 1). These structures were less often present in the balloons formed from salt chloroplasts than from sugar ones which were suspended in the same hypotonic solution.

In our sections through the balloons, its envelope was surely the single layer and the grana discs adherring to the surface of the balloon envelope were frequently

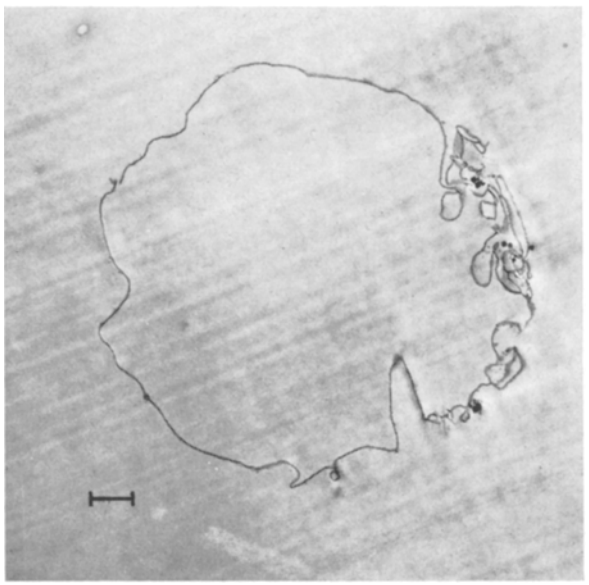

Fig. 1. Electron micrograph of the balloon-formation of salt chloroplasts in distilled water. The salt chloroplasts were prepared with 0.3 *M* NaCl-20 m*M* *Tris*-HCl (pH 7.5). × 6500. Marker indicates 1 μ. Condition; fixation in 6.5% glutaraldehyde buffered with 50 m*M* phosphate (pH 7.0) for 1 h. After washing in phosphate buffer, postfixation in 2% $KMnO_4$. Dehydration in acetone and embbeding in Epon 812.

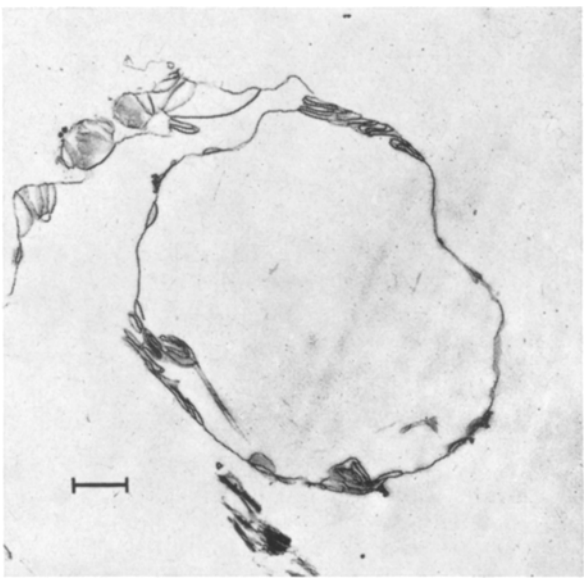

Fig. 2. Balloon-formation of sugar chloroplasts in 0.2 m*M* $MgCl_2$. The sugar chloroplasts were prepared with 0.4 *M* sucrose-20 m*M* *Tris*-HCl (pH 7.5). The grana discs adherring to the surface of the balloon are observed. × 7600.

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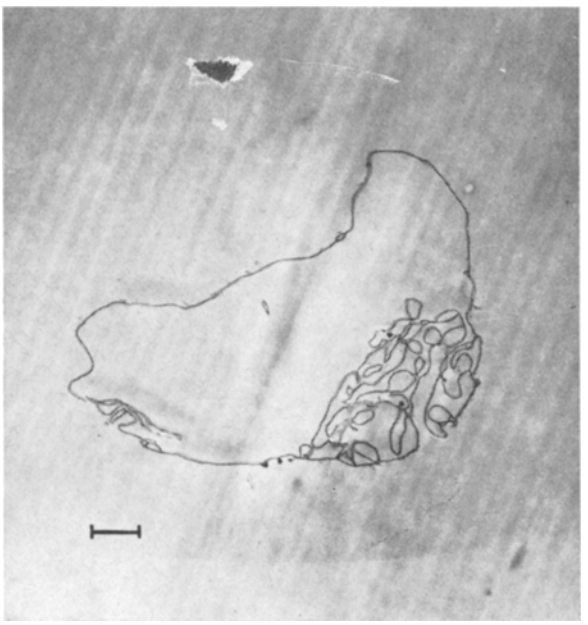

Fig. 3. Balloon-formation of salt chloroplasts in distilled water. The grana discs are so strongly distorted that individual discs are seldom recognizable and it is seen just like the unknitted configuration. × 6500.

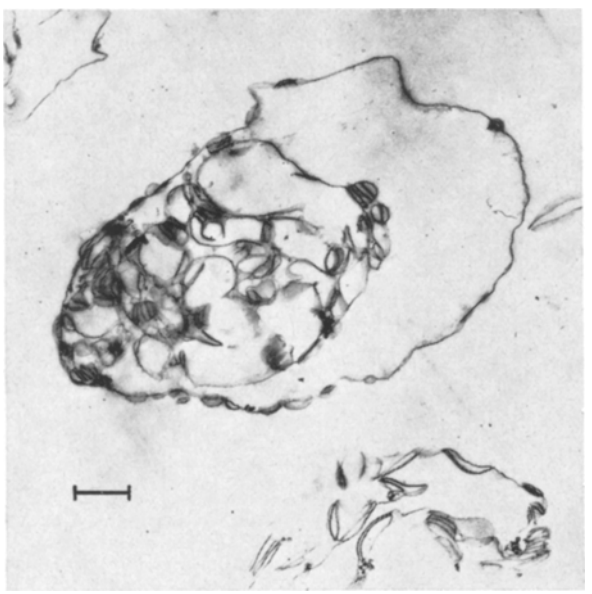

Fig. 4. Balloon-formation of sugar chloroplasts in 0.2 m*M* $MgCl_2$. The grana discs and vesicles are observed inside the balloon. × 7600.

observed (Figure 2). These observations clearly show that the balloon-formation results from the extension of stroma lamellae of chloroplasts. The tangled structure (Figure 3), which is represented the unknitted configuration, may be formed from the further swelling of the grana discs adherring to the balloon surface.

The explanation of the formation described above is based on the general model of lamellae structure that grana discs are stacked on the stroma lamellae. On the basis of this model, however, it cannot be explained from the balloon-formation how vesicles or grana discs are frequently observed inside the balloon (Figure 4). MENKE[11] reported that thylakoid stack may be formed by invagination, and WEHRMEYER[12] showed that at the edge of a granum, a lamellae may bifurcate or fold back on itself, thus contributing 2 discs to the same granum. Furthermore, WEIER et al.[13] showed the three-dimensional model of a single fret connected with several adjacent loculi. From the model of lamellae system as described above, it is not to be understood how the vesicles or grana discs observed inside the balloon are formed.

Although there have been electron microscopic investigations[6–8] on structural changes of isolated chloroplasts which were suspended in distilled water or hypotonic solutions, they have not observed the balloon as shown in our experiments, but separate small vesicles, separately swollen grana discs, swollen grana-fretwork system, irregular stroma lamellae lacking the grana discs, or swollen chloroplasts. In our observations, we never saw swollen grana-fretwork system[7] and irregular stroma lamellae lacking the grana discs[6,8]. The swollen grana-fretwork system shown with photomicroscope[13] is probably identical with the blebs formed in 0.05–0.1 *M* sucrose as reported by SPENCER and WILDMAN[3].

Zusammenfassung. Die Ballonbildung isolierter Spinatchloroplasten wurde elektronenoptisch untersucht.

S. HOSHINA and K. NISHIDA[14]

Botanical Institute, Faculty of Science, Kanazawa University, Kanazawa (Japan), 4 May 1970.

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Acoustic Stimulus Perception by the American Lobster *Homarus americanus* (Decapoda)

Crustaceans have been used extensively for physiological studies of vision and the nervous system[1], less so for the auditory system. LAVERACK[2] studied *Homarus* and determined the physiological sensitivity of hair-fan organs to various low frequency water vibrations. Drops of water and a moving diaphragm in the end of the test tank were two of the stimuli used. Recordings were made from nerves leading from hair-fan organs on the chelipeds and carapace. COHEN[3] found that one type of receptor in the statocyst of *H. americanus* responded to substratum vibration but not to air- or water-borne vibrations. A frequency response curve for substratum vibrations has been determined for the crab *Uca* with an unconditional response[4]. There have been a few reports of responses to sound stimuli by crustaceans[5].

In these studies there have been few adequate behavioral measures of what stimuli crustacea are able to perceive. It is hoped that the present technique of con-