

ELECTRON MICROSCOPIC EVIDENCE FOR THE LOCATION AND AMOUNT OF ION ACCUMULATION BY SPINACH CHLOROPLASTS

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INTRODUCTION

Light-induced ion uptake by spinach chloroplasts has been demonstrated by Nobel and Packer (1) using radioisotopes. An interdependent uptake of calcium and phosphate was observed under conditions favoring a light-triggered ATP hydrolysis. A light-induced uptake of sodium was found (1), which may occur by an exchange for potassium.¹ Mitochondria also manifest energy-dependent ion accumulation; deposits, presumably containing calcium, strontium, or barium, have been located within the mitochondria by electron microscopy (2-4). Recently, numerous electron-opaque particles were observed on the lamellae of spinach chloroplasts under conditions for strontium uptake (5). These particles increased in mean size as the illumination period was prolonged and were nearly absent in the dark.

The present report compares the electron-opaque particles on chloroplast lamellae in the presence of barium, calcium, and strontium, and shows as well the effect of illumination time on the size distribution of particles observed with barium. An attempt to locate sodium deposits is mentioned.

MATERIALS AND METHODS

Chloroplasts were isolated from spinach (*Spinacia oleracea*) in 175 mM NaCl, 50 mM Tris-HCl (pH 7.9) as previously described (5). The incubation medium consisted of the isolation medium plus 5 mM MgCl₂, 3 mM ATP, 10 μM N-methylphenazonium methosulfate, chloroplasts amounting to 100 μg chlorophyll/ml, and other additions as indicated. Incubation of 5-ml aliquots was at 25°C in 30,000 lux provided by tungsten lamps or in the dark. For studies on sodium localization, potassium salts replaced all sodium salts. The procedure for electron microscopy was as described

(5), except for sodium studies where no glutaraldehyde was added and the buffer for resuspension was 50 mM Tris-HCl (pH 7.4) containing 1% OsO₄ and 0.1% (2 mM) potassium pyroantimonate. The potassium salt of pyroantimonate is much more soluble than the sodium salt. Komnick (6) exploited this solubility difference to locate sodium deposits by electron microscopy, and this technique was employed here.

RESULTS AND DISCUSSION

When 1 mM BaCl₂ was added to the reaction mixture, many electron-opaque particles were observed on the chloroplast lamellae after a 30 min illumination (Fig. 1). Very few such particles were evident in the dark (7). When barium was omitted, no electron-opaque particles were found. These characteristics agree with observations for strontium or calcium additions (5).

The size distribution curve of electron-opaque particles in the presence of 1 mM BaCl₂ shifted to larger diameters as the illumination time was increased from 8 to 30 min (Fig. 2). After an 8 min incubation in the light, the mean particle diameter was 10.9 mμ, whereas after 30 min it was 13.3 mμ. Even after the 30 min illumination, the mean particle size with barium was smaller than that for a 15 min incubation with calcium or strontium. Electron-opaque particles observed with 1 mM CaCl₂ had a mean diameter of 14.3 mμ after a 15 min illumination (Fig. 2). For the same incubation except with 1 mM SrCl₂, the particles were larger than for calcium and the mean diameter was 20.6 mμ (Fig. 2).

The number of electron-opaque particles per unit area of lamellae was determined for various conditions. Area was measured by tracing single lamellae lying in the plane of the electron micrographs (final magnification 50,000) and weighing

¹ Nobel, P. S., data submitted for publication.

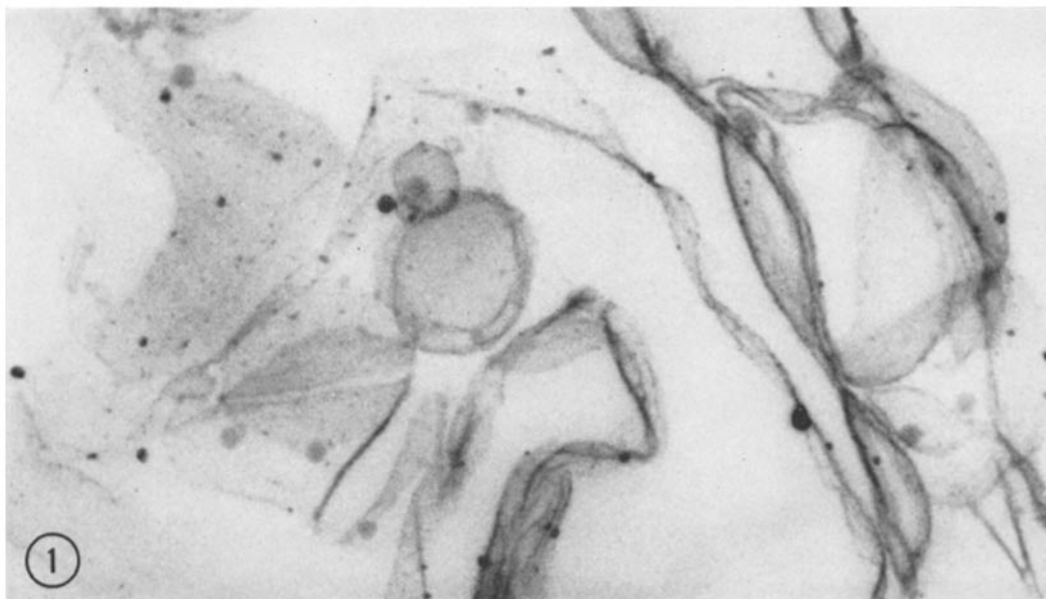


FIGURE 1 Electron-opaque particles on lamellae of spinach chloroplasts illuminated for 30 min in the presence of 1 mM BaCl₂. × 50,000.

the paper so circumscribed. For a 30 min illumination with 1 mM BaCl₂, 107 ± 5 (SD) particles were found per μ^2 of lamellae. For a 15 min illumination with 1 mM CaCl₂ or SrCl₂, 67 ± 4 or 108 ± 5 electron-opaque particles, respectively, were obtained per μ^2 of lamellae. In experiments with radioisotopes, 4.2 times more strontium than calcium was taken up by the chloroplasts (see reference 5). If the uptake were proportional to the mean diameter of the electron-opaque particles cubed times their frequency on the lamellae, then 4.7 times more strontium than calcium would be taken up. Therefore, the greater uptake of strontium than calcium may be due to both the size of the particles and their frequency.

Some ambiguity is involved in deciding on what side of the lamellae the electron-opaque particles are located. However, when short illumination times are used (2–8 min), the grana stacks are not so extensively swollen (5, 7) and the inner and outer surfaces of the thylakoid membrane can be determined. No evidence for deposits on the outside of the thylakoid membrane was obtained, but numerous instances of particles on the inner surface of the thylakoid membrane were found. Apparently, the ions penetrate the thylakoid membrane. The location of the deposits may explain

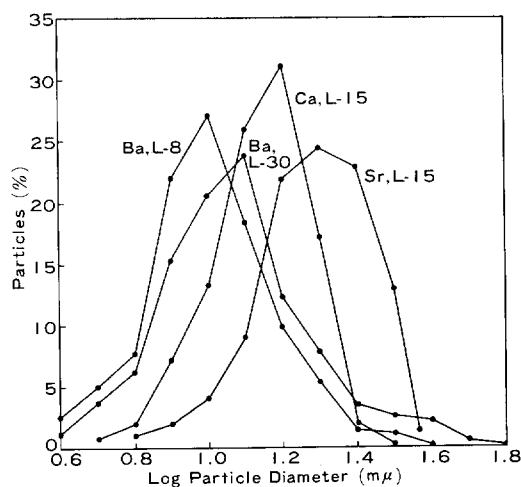


FIGURE 2 Size distribution of electron-opaque particles on chloroplast lamellae. Particle size was determined on photographs at × 50,000. For 1 mM BaCl₂, 1000 particles were measured on 18 photographs for an 8 min illumination (Ba, L-8), and on 19 photographs for a 30 min illumination (Ba, L-30). For 1 mM CaCl₂, 250 particles were measured on 4 photographs for a 15 min illumination (Ca, L-15). For 1 mM SrCl₂, 200 particles were measured on 6 photographs also for a 15 min illumination (Sr, L-15).

why appreciable ion uptake occurs only under conditions supporting chloroplast swelling.¹ The thylakoids progressively swell from a layered state of opposed membranes to a roundish vesicle-like structure as the illumination time is increased (5). This would expose sites on the inner surface of the thylakoid membrane to the reagents. Also, deposit growth would not be hindered by opposed membranes.

To study sodium localization, chloroplasts were incubated for 30 min in the presence of 3 mM NaCl, and potassium pyroantimonate was added after centrifugation. Control experiments using ²²Na showed that the uptake of sodium in the light was 2.5 μmoles/mg chlorophyll in 30 min. In the presence of potassium pyroantimonate, electron-opaque areas were observed in all electron micrographs. However, no deposits dependent on added sodium could be identified in the light or dark, even when the pyroantimonate concentration was increased to 2%. One reason may be the relative ease with which sodium is washed out of chloroplasts. For example, if the chloroplast pellet after a 30 min illumination is resuspended in 5 ml of the isolation medium and then recentrifuged, 83% of the accumulated sodium is washed out whereas only 36% of the accumulated strontium is removed from the pellet. On the other hand, different mechanisms are indicated for the uptake of monovalent compared to bivalent cations. Calcium uptake can be increased by added phosphate (1), suggesting that the electron-opaque particles may contain a phosphate. However, sodium uptake is not enhanced by added phosphate (1); instead, sodium uptake occurs concomitantly with a potassium release¹ and the formation of deposits may not be involved.

X-ray diffraction was performed with samples incubated in the light for 30 min with 1 mM CaCl₂ or SrCl₂. After incubation and centrifugation, the chloroplast pellets were washed twice with 10 ml of distilled water and three times with 10 ml of acetone. 55% of the accumulated strontium was

retained after this procedure while soluble ions would be diluted 4×10^6 times. No evidence of crystals attributable to calcium or strontium salts was found by X-ray diffraction. Also, electron diffraction studies of the particles on the chloroplast lamellae have failed to reveal crystals (5). Hence, the deposits appear to be amorphous.

SUMMARY

Electron-opaque particles occur on the lamellae of spinach chloroplasts under conditions favoring the uptake of certain bivalent cations. These particles are apparently amorphous deposits which increase in size during illumination. The mean diameter is largest with strontium (20.6 mμ for a 15 min illumination), intermediate with calcium, and smallest with barium. The frequency is about 100 deposits per μ² of lamellae. The electron-opaque particles are located on the inner surface of the thylakoid, stressing the difference between the two sides of the membrane.

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