The Effect of Air Ions on Light-Induced Swelling and Dark-Induced Shrinking of Isolated Chloroplasts

by

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INTRODUCTION

The swelling of chloroplasts in response to light has been recognized for many years. In recent microscopic studies we have observed that isolated chloroplasts shrink when stored for 48 hr at 4°C and swell again when illuminated (Kotaka, Krueger and Andriese, 1967). The capacity of the chloroplasts to shrink and swell was well preserved in a medium which will be described later. Swelling of 80 to 90% of the chloroplasts occurred during illumination for 2 hr with 5,000 ft-c at 4°C. Subsequent storage for 12 hr at 4°C in the dark produced shrinking which again was reversed by illumination. The rates of swelling and shrinking (designated as s-s) were inhibited by prolonged illumination or by the addition of NaF. We further observed that incubation of the chloroplasts at 4°C in the dark or in the light while exposed to unipolar ionized air of either charge produced a measurable increase in s-s activity.

In order to obtain some clue to the mechanisms involved in this air-ion-enhanced s-s effect, the experiments described in this paper were performed.

MATERIALS AND METHODS

CHLOROPLAST PREPARATION.- Turgid leaves of spinach (SPINACIA OLERACCA L.) were selected. Washed leaves (100 g) were refrigerated for at least one hour. The leaves were cut into small pieces and homogenized in a Waring Blender for 10 sec with 300 ml of isolation medium containing 0.7 M sucrose, 0.01 M EDTA-2 Na, 0.002 M cysteine-HCl, 0.1% Merthiolate® (sodium ethylmercurithiosalicylate) in 0.2 M tris-HCl buffer pH 7.2. The homogenate was then filtered through 4 layers of cheese cloth, and the filtrate was centrifuged at 200 x g for 10 min. The supernatant was recentrifuged at 2,500 x g for 10 min.

The residue was resuspended in the isolation medium, and the double centrifugation procedure was repeated. Finally, the chloroplasts were suspended in the same medium to give chlorophyll concentrations of about 1.5 mg/ml (stock suspension). Chlorophyll was determined by the method of MacKinney, modified by Vishniac (1957).

EXPOSURE TO UNIPOLAR AIR IONS.- One hundred ml aliquots of chloroplast suspension were placed in the ion-exposure chambers (Kotaka, Krueger and Andriese, 1965). The air ion densities were: 2.5 x 10^5 negative ions/cm³ of air, 3.1 x 10^5 positive ions/cm³ of air in the negative and positive chambers respectively. No air ions of opposite sign were detected in either chamber. In the control chamber, the ion density was ca. 500 ions/cm³ each of negative and positive ions.

The chloroplast suspension in each chamber was incubated at 4°C with or without illumination, as specified. The light used was emitted from fluorescent lamps and...
the light intensity was 5,000 ft·c at the surface of the incubation medium.

DETERMINATION OF SHRINKING AND SWELLING OF CHLOROPLASTS.—At stated intervals, the variously treated chloroplasts were collected by a capillary pipette and transferred to a slide. A cover slip was laid down and held in place with vaseline at two opposite edges. In order to count the number of chloroplasts, the hemocytometer was used.

DETERMINATION OF LIGHT-SCATTERING.—Light-scattering studies were carried out by the method developed by Packer and Marchant (1964). The chloroplast samples to be assayed were collected at stated intervals and were suspended in a medium containing Tris buffer (0.02 M, pH 8.0), NaCl (0.055 M), KH$_2$PO$_4$ (4 mM), MgCl$_2$ (5 mM), and freshly prepared sodium ascorbate (750 μM). The final chloroplast concentration was equivalent to 3 μg of chlorophyll per ml. Light-scattering changes produced by the chloroplasts were determined at 90°C in a Brice-Phoenix light-scattering apparatus. Incident and scattered light were filtered at 546 mμ. The scattering was adjusted at the outset to read 100% by using the minimum intensity of light at 546 mμ. It is established that light in this region is close to the minimum of the photochemical action spectrum. Changes in the scattered light intensity in response to actinic red light (6 x 10$^{16}$ quanta/sec in the range from 600 to 700 m/μ) are recorded in terms of percentage change from the initial scattering level. The temperature of the system was controlled during the period of measurement at 25 ± 0.1°C by circulating liquid around the jacketed cuvette (1 x 1 cm).

DETERMINATION OF ADENOSINE TRIPHOSPHATASE ACTIVITY AND PHOTOPHOSPHORYLATION.—The assay methods developed by Packer and Marchant (1964) were adopted with slight modifications. The reaction system consisted of 50 mM tris buffer, pH 8.0, 20 μM of phenazine methosulfate or flavin mononucleotide, 0.5 mM of sodium ascorbate, and 5 mM of MgSO$_4$. The following substrates were added to the system separately: 5.0 mM of ATP for hydrolysis and 2 mM each of ADP and KH$_2$PO$_4$ for synthesis determination. Both hydrolysis and synthesis of ATP were measured over a 20-min period, starting 30 sec after the addition of ATP or ADP respectively, by determining the change in the phosphate concentration of the reaction system using Horwitt's phosphomolybdic acid method (Horwitt, 1952).

The assays of adenosine triphosphatase activity and phosphorylation were performed in test tubes placed in a thermostatic bath at 27°C under constant illumination. A 150-watt General Electric reflector flood bulb was used to illuminate the reaction mixture. The light intensity was 5,000 ft·c at the surface of the test tube.

RESULTS AND DISCUSSION

After 1-hr incubation at 4°C in the dark, chloroplasts had a contracted and elongated disk-like appearance. Their granulated structures (grana) were barely discernible. When these disk-like chloroplasts were illuminated, most of them (80 to 90%) swelled into spherical shapes and their grana structures became clearly visible. In this paper we define the "shrunken" chloroplasts as those which have a contracted and elongated disk-like appearance with hardly visible grana, and the "swollen" chloroplasts as those which have a swollen spherical appearance with clearly visible grana. The average dimensions of shrunken chloroplasts were 7.3 μ x 0.5 μ, and those of swollen chloroplasts were 8.0 μ x 8.0 μ.

Shrinking and swelling of chloroplasts were clearly observed microscopically. The rate of shrinking and swelling activity increased when ATP was added to the chloroplast suspension on the slide. A similar increase in the s-s activity of chloroplasts was observed when the preparations were exposed to air ions of either charge. These observations led us to infer that air ions stimulate the swelling-shrinking activity of chloroplasts.

In order to obtain quantitative confirmation of our microscopic observations of the s-s phenomenon, further experiments were conducted using the light-scattering method for measuring swelling and shrinking of the chloroplasts.