

Investigations on Ion Fluxes of Chloroplasts with an Intact Envelope

R. Pflüger

Botanisches Institut der Tierärztlichen Hochschule,
Abteilung Biochemie der Pflanzen, Hannover

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During illumination of isolated broken chloroplasts electron transport dependent proton-uptake into the thylacoid space takes place¹. According to the chemiosmotic theory the pH-gradient forces photophosphorylation². H⁺-uptake is electrically compensated by an efflux of Mg²⁺ and K⁺ in a nearly stoichiometric ratio³. Recently with whole intact chloroplasts such H⁺-fluxes across the thylacoid membrane could be demonstrated, too⁴.

The intact chloroplast is surrounded by two membranes. The outer membrane is readily permeable even for larger molecules, whereas the inner membrane is the functional barrier between the cytoplasm of the cell and the stroma of the chloroplast⁵. As was shown by Heber and Krause⁶ there is no H⁺-exchange across this membrane. The present paper deals with the problem, whether this is valid for Mg²⁺ and K⁺, too. The result would be of particular importance in respect of a possible regulation mechanism of Mg²⁺- or K⁺-dependent enzymes of the Calvin-cycle^{7,8}. A rapid exchange of these cations between chloroplast and cytoplasm would largely abolish the primary increase of Mg²⁺- and K⁺-concentrations in the stroma during illumination.

Thus, light-dependent extrusion of Mg²⁺ or K⁺ out off the thylacoid would be useless as a factor of enzyme regulation.

The isolation of intact spinach chloroplasts was performed according to the method of Jensen and Bassham⁹. The translocation of K⁺ and Mg²⁺ were followed by measuring the steady state-concentrations of the suspending medium in the dark and light. For this purpose, the chloroplasts were rapidly separated from the incubation medium. A filtration through millipore filters was applied by which about 400 µl particle free supernatant were obtained within 5 sec. In the supernatant the cations were measured by atomic-absorption-spectrophotometry.

Both the broken and the intact chloroplast preparations exhibit an efflux of K⁺ and Mg²⁺ in the medium upon illumination (Table I). Nevertheless, the changes are smaller in the preparation of the intact chloroplasts. However, this preparation contains only a certain portion of intact chloroplasts which can be determined by the ferricyanide-method, developed by Heber and Santarius¹⁰. Ferricyanide is rapidly reduced by broken chloroplasts, whereas the envelopes of intact chloroplasts prevent the permeation of ferricyanide. After correction by the portion of broken chloroplasts present in the preparation the intact chloroplasts do not cause an increase of Mg²⁺- and K⁺-concentrations in the medium upon illumination. The result indicates that the chloroplast envelope is not readily permeable for these two cations.

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Table I. Comparison of Mg²⁺- and K⁺-translocations of broken and intact chloroplasts. The assay medium (4 ml) contained 0.38 M sorbitol for intact and 0.05 M sorbitol for broken chloroplasts. Additionally, both media contained 30 µM PMS and 0.2 mM MES pH 6.5. The concentration of chloroplasts was 0.049 mg chlorophyll/ml. After an incubation of 2 min at 20 °C the dark-sample, after 1 min red-light (RG 630 Schott-Filter, 8 × 10⁵ ergs/cm·sec) the light-sample were taken. The portion of intact chloroplasts of the "intact preparation" was determined after the ferricyanide-method by Heber and Santarius¹⁰. Rates of ferricyanide-reduction (µmole FeCy red./mg chl.·h) : broken chloroplasts: 301 (100.0%), intact chloroplasts: 121 (40.2%).

Chloroplasts	Steady state K ⁺ -concentration in the medium (µM)		Light-dark (change in nmole K ⁺ /mg chl. translocated into the medium)	Steady state Mg ²⁺ -concentration in the medium (µM)		Light-dark (change in nmole Mg ²⁺ /mg chl. translocated into the medium)
	dark	light		dark	light	
broken	90.5	101.2	+ 220	63.5	67.3	+ 75
"intact"	88.2	92.0	+ 80	46.8	48.2	+ 30
intact _{corrected}			— 8			± 0

Requests for reprints should be sent to Dr. Reinhard Pflüger, Botanisches Institut der Tierärztlichen Hochschule, D-3000 Hannover-Kirchrode, Bünteweg 17 d.



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