Investigations on Ion Fluxes of Chloroplasts with an Intact Envelope

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During illumination of isolated broken chloroplasts electron transport dependent proton-uptake into the thylacoid space takes place [1]. According to the chemiosmotic theory the pH-gradient forces photophosphorylation [2]. H^+-uptake is electrically compensated by an efflux of Mg^{2+} and K^+ in a nearly stoichiometric ratio [3]. Recently with whole intact chloroplasts such H^+-fluxes across the thylacoid membrane could be demonstrated, too [4].

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 chloroplast [5]. As was shown by Heber and Krause [6] there is no H^+-exchange across this membrane. The present paper deals with the problem, whether this is valid for Mg^{2+} and K^+, too. The result would be of particular importance in respect of a possible regulation mechanism of Mg^{2+}- 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 Mg^{2+}- and K^+-concentrations in the stroma during illumination. Thus, light-dependent extrusion of Mg^{2+} 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 Bassham [9]. The translocation of K^+ and Mg^{2+} 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 supernant were obtained within 5 sec. In the supernant the cations were measured by atomic-absorption-spectrophotometry.

Both the broken and the intact chloroplast preparations exhibit an efflux of K^+ and Mg^{2+} 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 Santarius [10]. 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 Mg^{2+}- and K^+-concentrations in the medium upon illumination. The result indicates that the chloroplast envelope is not readly permeable for these two cations.

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Table I. Comparison of Mg^{2+}- 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, 8×10^5 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 Santarius [10]. 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

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