LIGHT-INDUCED HIGH-AMPLITUDE SWELLING OF SPINACH CHLOROPLASTS

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Chloroplasts *in vitro* manifest two types of mechanisms controlling their structure. There is an osmotic mechanism, which results in changes in chloroplast volume in response to changes in tonicity of suspending media, and a light-dependent mechanism. Nishida (1) has described the passive osmotic swelling of spinach chloroplasts in sucrose solutions by optical, gravimetric, and volumetric techniques. The light-induced changes in chloroplast structure that have been observed by light-scattering (2), and by the Coulter Counter and electron microscopy (3) lead to shrinkage. This action of light brings about low-amplitude volume changes in chloroplasts by energy-dependent mechanisms; shrinkage occurs under conditions of electron transport, light-triggered ATPase, or both (4) and is characterized by being rapid and reversible (with half-times for their growth in light and decay in dark of about 20 seconds).

Chloroplasts and mitochondria resemble one another in that both systems manifest osmotic and low amplitude volume changes (5,6). Moreover, mitochondria show a deteriorative type of swelling change that is dependent upon electron transport (5). High amplitude volume changes occur much more slowly than the low amplitude type and are accompanied by extensive changes in mitochondrial structure and organization. This type of swelling change has not yet been described for chloroplasts. The close correspondence of the response of chloroplasts and mitochondria with regard to structural changes suggested that chloroplasts might manifest a similar light-induced, high-amplitude swelling process. Accordingly, chloroplasts were isolated from spinach leaves, and use was made of the simple absorbancy measurements that Nishida has employed for assessing swelling (decreased absorbancy). Figure 1 shows a time recording of the extinction changes at 540 mμ of chloroplasts suspensions incubated in the light or in the dark. In the light the optical changes

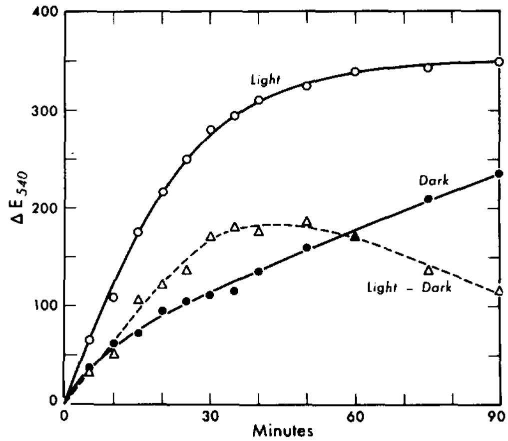

Figure 1. Light-induced absorbancy decreases in spinach chloroplasts. Chloroplasts were isolated as previously described (4) in Tris-HCl (100 mM at pH 7.6) - NaCl (350 mM). The reaction mixture contained 20 mM Tris-HCl (at pH 8), 350 mM NaCl, 20 μM phenazine methosulfate (PMS), and chloroplasts (16 μg chlorophyll/ml). The absorbancy of the chloroplasts was 735 at zero time; results are given as the change in absorbancy (ΔE) during the incubation period. Tubes containing the reaction mixture were kept in a water bath at 25°C. Dark tubes were wrapped in aluminum foil; light tubes received 25,000 lux from a 150 watt reflector flood lamp.

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are much larger than in the dark and reach a maximum after about 60 minutes, but in the dark continue to increase even up to 90 minutes. The dashed curve is the light minus dark plot, which shows a maximum after incubation for 30 minutes.

To confirm if absorbancy measurements are a reliable indicator of high-amplitude volume changes, a comparison was made between several methods for assessing chloroplast swelling. It was found that light induces about a two-fold increase in the packed volume and wet weight of the chloroplast pellets formed in "chlorocrit" tubes after incubation under conditions that result in absorbancy decreases. When volume changes are assessed with the Coulter counter, the slow speed and high amplitude of the light-induced swelling is confirmed (fig 2). Hence, this comparison of methods is the definitive proof that the absorbancy decreases observed by Nishida (1) affords a measure of chloroplast swelling. Some other pertinent observations are: 1) that the initial absorbance of chloroplast suspensions is determined by the tonicity of the suspending medium, e.g. NaCl concentration, resulting from osmotic volume changes but 2) that light induces swelling as judged by absorbancy decreases

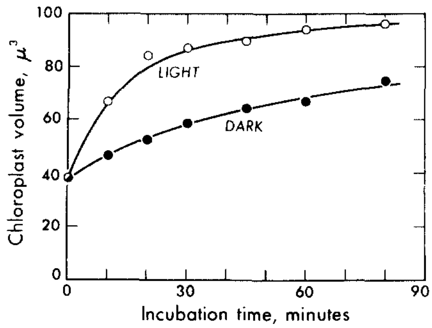

Figure 2. Light-induced Volume Changes in Spinach Chloroplasts Determined by a Coulter Counter. Chloroplasts were isolated in 175 mM NaCl, 50 mM Tris-HCl (pH 8) and the experiment performed in the same medium. The first centrifugation was for one minute at 200 g, with the supernatant being spun for ten minutes at 200 g to form a chloroplast pellet. Incubation conditions as in fig 1. Samples were diluted 2×10^3 immediately before plotting the volume distribution using a 100 μ orifice. The chloroplast volume is defined as the total volume of all particles larger than 12 μ^3 divided by the number of such particles.

TABLE I

Activation and Inhibition of High-Amplitude Chloroplast Swelling

The reaction mixture contained: 20 mM Tris-HCl (at pH 7), 350 mM NaCl and chloroplasts (chlorophyll 17.6 μg/ml), incubation was for 20 minutes. Additions were 20 μM for PMS, 10 mM for phosphate (P_i) and 2 mM for NH_4Cl.

	% Decrease E_{540}	
	Light	Dark
no addition	16.3	8.2
PMS	39.5	0.8
P_i	6.9	3.9
NH_4Cl	13.3	0.8
PMS + P_i	26.8	3.9
PMS + NH_4Cl	39.8	0.8
PMS + NH_4Cl + P_i	33.8	0.8
NH_4Cl + P_i	10.6	0.0

and packed volume increases in a range of NaCl concentration between 0.1 - 1.0 M that is approximately the same on a percentage basis.

The action of some activators and inhibitors of high-amplitude chloroplast swelling is given in Table I. A "spontaneous" chloroplast swelling occurs in the dark. In the absence of added cofactors, light induces an enhancement of swelling (Table I and fig 2). It has been found (during a survey of photophosphorylation cofactors and inhibitors) that PMS stimulates the extent of swelling in the light but not in the dark. Table I shows that a powerful inhibitor of the light-induced high-amplitude swelling is inorganic phosphate. This observation has an important bearing upon the relationship (to be reported elsewhere) between low-amplitude volume changes in which light produces shrinkage and the light-induced high amplitude swelling. An ATP-linked contraction of high-amplitude swelling, which occurs in mitochondria has not been observed in chloroplasts. Other results show that $MgCl_2$, ADP, and NH_4Cl (Table I) do not affect high-amplitude chloroplast swelling. Therefore, this process appears light-, but not energy-dependent, whereas low-amplitude volume changes are both light and energy dependent and are affected by these reagents (2-4).

It is of considerable interest that the existence of high-amplitude volume changes in chloroplasts dependent upon light (i.e., electron transport) could be predicted from mitochondrial research. Since this type of swelling in mitochondria is associated with deterioration, this process may be correlated with a destruction of structure during aging of chloroplasts _in vitro_, and by extension, in the living cell.

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