OSMOTIC AND LIGHT-INDUCED VOLUME CHANGES IN CHLOROPLAST MEMBRANE FRAGMENTS

Elizabeth Gross and Lester Packer
Department of Physiology, University of California, Berkeley, California

Received August 9, 1965

Isolated chloroplasts manifest structural changes upon illumination as measured by 90° light-scattering or absorbancy (1,2,3). These effects produced by actinic light have been correlated with volume changes by Itoh, Izawa, and Shibata (4), and others (5,6) in spinach chloroplasts. Osmotic volume changes of spinach chloroplasts in vitro have also been observed by Nishida (7). It has also been observed that chloroplast membrane fragments (8) and chromatophore preparations from Rhodospirillum rubrum show light-scattering changes similar to those seen for whole chloroplasts (9). These observations suggested that membrane fragments may also possess the ability to undergo volume changes, and that such phenomena may collectively account for the observed volume changes of intact chloroplasts. Hence an investigation was made to determine whether fragments of chloroplast membranes can undergo volume changes by osmotic and light-induced mechanisms.

METHODS

Preparation of membrane fragments - Chloroplasts were isolated from washed spinach leaf material in NaCl (175 mM) and Tris-HCl (50 mM, pH 8), and fragments prepared by sonication of suspensions for 20 sec at 20 kc/sec. Fragments of different size classes were separated by differential centrifugation for 10 minutes at 5-10,000 x g, 10-20,000 x g or 20-40,000 x g. These fractions were resuspended in the isolation medium and then passed through a 0.8 μ millipore filter to eliminate large particles.

Osmotic volume measurements - Osmotic properties were examined by packed volume, packed wet mass, and absorbancy measurements under the general conditions indicated below with the specific details given with the individual experiments. Packed volume was measured after centrifugation of a suspension of fragments for 1 hr at 13,000 x g in a centrifuge adapted for capillary tubes. Packed wet mass determinations for the 5-10,000 x g fraction were made by weighing pellets obtained by centrifugation for 30 min at 20,000 x g. The absorbancy measurement was a modification of the method of Nishida (7); namely, the ratio of the absorbancy 680:546 mμ was taken as a measure of osmotic volume. Osmolarities were

determined by freezing point depressions.

Volume changes produced by illumination - Illumination of chloroplasts is known to produce two types of volume changes - a rapid shrinkage and a slow swelling. The circumstances under which these phenomena were measured were substantially as previously described by Packer et al (5). Swelling was measured by following absorbancy decreases at 546 mμ of chloroplast or fragment suspensions after incubation in darkness (controls) or under illumination with actinic light. Shrinkage was measured by following either 90° light-scattering increases at 546 mμ, or decreases in packed volume (5) or wet mass of illuminated fragments. In order to measure the packed volume decreases, the metal cover of the capillary centrifuge was replaced by lucite to permit illumination during centrifugation. For packed wet mass measurements, the fragments were illuminated for 10 minutes prior to centrifugation for 10 minutes at 60,000 x g after which the pellets were weighed.

RESULTS

Osmotic properties - The volume of osmotically active particles obeys the relation $V = V_\infty + K/C$: where C is the total osmolarity of the suspending medium; V is the volume of the particles; V_∞ their volume at infinite solute concentration (or the osmotically inactive volume): $V - V_\infty$ is the osmotically active volume; and the constant K is the rate of change of the volume with reciprocal osmolarity (10). A test for osmotic properties of chloroplast fragments in various solutes is shown in table I. Since the values for V_∞ and K for 5-10,000 x g fragments are very similar for NaCl and three non-electrolytes, the volume of fragments seems to depend upon the number of solute particles.

Since osmotic volume changes result from a variation in the water content of the particles, a change in mass should accompany changes in osmolarity according to the formulation $M = M_\infty + K'/C$: where M = mass; M_∞ = mass at infinite solute concentration (i.e. mass of dry solutes plus bound water); C = the osmolarity of the medium; and K' is a constant for the rate of change of mass (water content) with reciprocal osmolarity. Table I shows close correspondence between K' and M_∞ in sucrose or NaCl.

Absorbancy measurements offer strong corroborative evidence in any test for osmotic properties because rapid measurements in dilute solutions can be made. According to Tedeschi and Harris (10) $\frac{1}{E} = \beta + K''/C$, where K" is a constant, C is as above. Note that $\frac{1}{E}$ is the reciprocal of absorbance at a particular wavelength, and $\frac{1}{E} - \beta$ is proportional to the osmotically active volume ($V - V_\infty$). Since absorbance of chloroplast fragments at 680 mμ (the red chlorophyll absorption peak) is independent of osmolarity and reflects the number of particles in the solutions, a test for osmotic properties in various solutes (figure 1) can be provided by measuring the ratio of 680:546 mμ absorbancy. Similar osmotic

TABLE I

Evidence for Osmotic Properties in Chloroplast Membrane Fragments

Solute	V_∞ (μl pellet/mg chlorophyll)	K (μl pellet/mg chlorophyll/osmole)
Packed volume[a]		
NaCl	79	4.1
Sucrose	88	3.7
Glucose	87	3.6
Raffinose	92	4.2
Packed wet mass[b]		
Solute	M_∞ (mg pellet/mg chlorophyll)	K' (mg pellet/mg chlorophyll/osmole)
NaCl	74	2.7
Sucrose	85	3.0

[a] Packed volume $V = V_\infty + K/C$. V = the pellet volume. V_∞ is the osmotically dead volume. C = the total osmolarity of the suspending medium. K = the rate of change of volume with reciprocal osmolarity. Fragments suspended at 2 mg/ml chlorophyll in Tris-maleate (20 mM, pH 6.8) plus other solute were centrifuged for one hour at 13,000 x g in the "capillary" centrifuge.

[b] Packed wet mass. $M = M_\infty + K'/C$. M = the pellet mass. M_∞ is the pellet mass at infinite solute concentration (dry mass + bound water). C = total osmolarity. K' = the rate of change in mass (water content) with reciprocal osmolarity. Fragments suspended at 0.4 mg/ml chlorophyll in solutions of various osmolarities were centrifuged in 5 ml tubes at 20,000 x g for 30 min, after which the supernatant was discarded and the pellets were weighed.

behavior is observed in non-electrolytes: sucrose, mannitol, and maltose; raffinose and glucose (not shown). Also there was no systematic dependence in this experiment of absorbance upon the refractive index of the solute. For electrolytes, such as NaCl or KCl the intercept is displaced but the slope is similar to that for non-electrolytes. Glycerol, which is known to readily penetrate membranes, does not cause osmotic volume changes. Other experiments have shown that smaller fragments, eg. 10-20,000 and 20-40,000 x g fractions also manifest osmotic properties, but such responses decrease with particle size.

Volume changes of illuminated chloroplast fragments - Fragments also retain the ability to shrink and swell upon illumination (table II). A comparison of chloroplasts and fragments shows that the magnitude of the light induced response decreases with fragment size. Under normal conditions ($MgCl_2$ and Phenazine methosulfate present) the changes are reversible (i.e. the particles recover their initial volume when the light is extinguished. However in the presence of $MnCl_2$ alone, chloroplasts and fragments display a new type of light induced shrinkage which is irreversible in the dark and which is greater in the 5-10,000 x g

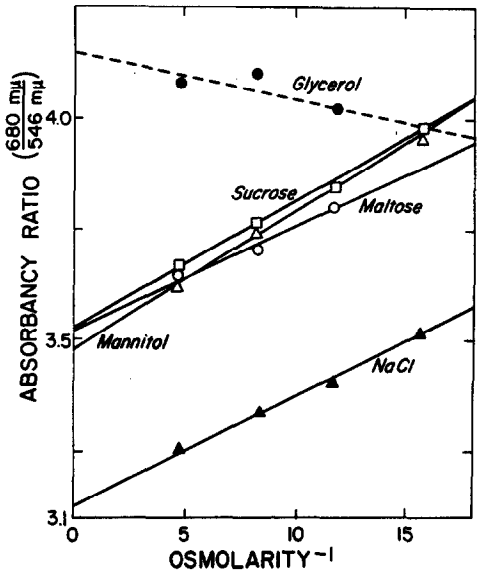

Figure 1. Osmotic response of 5-10,000 x g fraction of chloroplast fragments. Fragments were suspended in Tris buffer (20 mM, pH 6.8) plus other solutes. The absorbancy ratio, $680/546$ mμ = $\beta + K''/C$, β = the intercept at infinite solute concentration, C is the total osmolarity of the medium, and K" is the rate of change of the absorbancy ratio with reciprocal osmolarity.

TABLE II

Comparison of Light-Induced Structural Changes of Chloroplasts and Fragments

Fraction	Percent change upon illumination		
	Shrinkage[a]		Swelling[b]
	Reversible	Irreversible	
Chloroplasts	132	30	51
5-10,000 g fragments	69	66	16
10-20,000 g fragments	39	33	11
20-40,000 g fragments	15	19	1

[a] The percent increase in 90° light-scattering level at 546 mμ upon illumination with red actinic light was calculated as follows: (light-dark)/dark x 100. Reversible shrinkage occurs in the presence of Tris-maleate (20 mM, pH 6.8), $MgCl_2$ (5 mM), ascorbate (2.5 mM), PMS (20 μM), 5 μg/ml chlorophyll. Irreversible shrinkage in the presence of Tris-maleate (20 mM, pH 6.8), $MnCl_2$ (5 mM), and 5 μg/ml chlorophyll.

[b] Percent decrease in absorbancy at 546 mμ in the presence of NaCl (350 mM), Tris-HCl (40 mM, pH 7.5), PMS (20 μM) and chlorophyll (10 μg/ml). Illumination was 30 min.

fragments than in chloroplasts. Fragments also retain the ability to swell to some extent which decreases with the fragment size.

If size changes in fragments are true volume changes accompanied by water movement then decreased packed volume and mass should be observed under the shrinkage condition. Indeed evidence for volume decreases of about 20 percent in illuminated fragments, incubated in the presence of $MgCl_2$ and PMS, has been obtained. Also a decrease in water content of up to 15% of the pellet mass is observed when fragments are illuminated in the presence of $MnCl_2$.

DISCUSSION

The results of this investigation indicate that chloroplast membrane fragments undergo volume changes accompanied by water flow both in response to an osmotic gradient and in response to light. These findings are in agreement with the morphological observations of Weier et al (11) and Itoh et al (4) which show that chloroplasts contain subcompartment structures which change size in response to osmotic gradients or light respectively. Hence studies with isolated membrane fractions, reported here, suggest that grana or other subcompartments in the chloroplast may be responsible for structural changes that result in water movements of whole chloroplasts.

ACKNOWLEDGEMENT

This research was supported by the National Science Foundation (GB-1550) and a Public Health Service Fellowship (1-F1-GM-22,599-02) from the National Institutes of Health.

REFERENCES

1. Packer, L. Biochim. Biophys. Acta 75, 12 (1963).
2. Jagendorf, A.T. and Hind, G. in Photosynthetic Mechanisms of Green Plants, Nat'l Acad. of Sci., pub. 1145, Washington D.C. p. 599 (1963).
3. Dilley, R.A. and Vernin, L.P. Biochem. 3:817 (1964).
4. Itoh, M., Izawa, S., and Shibata, K. Biochim. Biophys. Acta 66, 319 (1963).
5. Packer, L., Siegenthaler, P.A., and Nobel, P.S. J. Cell Biol.26: August (1965).
6. Dilley, R.A. and Vernon, L.P. Arch. Biochem. Biophys. In press (1965).
7. Nishida, K. Plant and Cell Physiol. 4, 247 (1963).
8. Packer, L. and Marchant, R.H., J. Biol. Chem. 239:2061 (1964).
9. Packer, L., Marchant, R.H. and Mukohata, Y. Biochim. Biophys. Acta 75:23 (1965).
10. Tedeschi, H. and Harris, D.L. Arch. Biochem. Biophys. 58, 52 (1965).
11. Weier, T.E., Stocking, C.R., Bracken, C.E., and Risley, E.B. Amer. J. Botany 52, 339 (1965).