

THE INFLUENCE OF HEATING ON THE MORPHOLOGY AND  
PHOTOCHEMICAL ACTIVITY OF ISOLATED CHLOROPLASTSby Y.G.Molotkovsky and I.M.Zheskova,  
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There exists a close relationship between the morphological state and the activity of energy-transforming cell structures, mitochondria (Lehninger, 1962) and chloroplasts (Packer and Marchant 1964). Investigations of isolated mitochondria have shown that a suppression of their activity is accompanied by swelling and a loss by membranes of the properties of semipermeability. Recently a certain entity of processes has been established, which determine morphological changes in mitochondria and chloroplasts (Packer, 1963).

This study was undertaken to discover the influence of heating on the morphological condition and activity of isolated chloroplasts.

Methods and material. Chloroplasts were isolated from leaves in cold after Arnon et al. (1956). Swelling and contraction of chloroplasts were followed spectrophotometrically at 520 m $\mu$ . Chloroplast lipids were extracted according to Nichols (1963). Preliminary separation of lipids was effected by the method of thin-layer chromatography on silicic acid in a chloroform-methanol (9:1) solvent. The section of the chromatograms with a band of free fatty acids was eluted and repeatedly divided in a hexane-diethyl ether-acetic acid (70:30:1) solvent. The methyl ethers of the fatty acids were

separated on a Pay argon chromatograph (Great Britain).

Results and discussion. Heating of the suspension causes the chloroplasts to swell (Fig.I). The swelling can be inverted by adding ATP and  $Mg^{++}$  to the suspension. The effect of ATP is intensified by simultaneously adding serum albumin (SA). The action of ATP is momentary and in a few minutes the contracted chloroplasts begin to swell. A repeated addition of ATP to the incubation medium again results in a contraction superseded by swelling. The chloroplasts injured by heating restore their initial morphological state when there is an excess of ATP. As ATP is consumed, repeated swelling sets in.

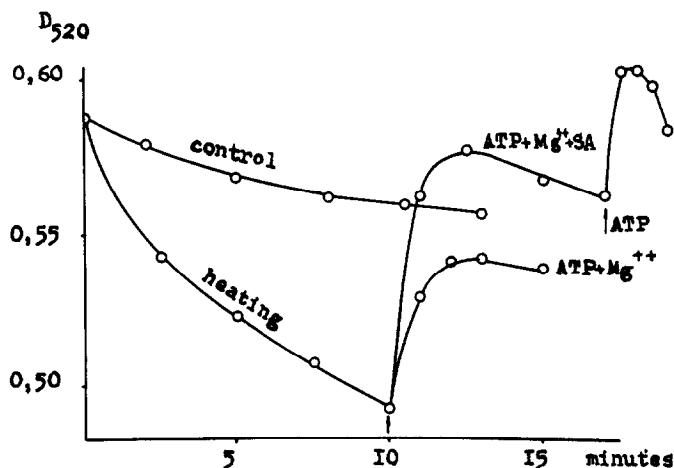


Fig.I. Swelling of chloroplasts under the influence of heating.

The medium contains NaCl (0.175 M), tris-HCl buffer pH 7.8 (0.05 M). Additions, as indicated by arrows, to a final concentration of ATP (5 mM),  $MgCl_2$  (2 mM) and SA (1 mg/ml). Temperature 40°C.

As result of heating, the activity of the Hill reaction drops abruptly (Table I). Such a suppression of photochemical

activity is inverted to a considerable extent by adding a mixture of ATP,,  $Mg^{++}$  and SA to the medium after heating.

Table I  
Effect of heating on the Hill reaction  
% of inhibition

Control	336	
Heating (15 minutes at 40°C)	25	92
Heating +ATP, $Mg^{++}$ , SA	254	24

Medium and additions as in Fig. I + ferricyanide (3  $\mu M$ ).  
Contents of chlorophyll 20  $\mu g/ml$ . Illumination 42500 lux,  
exposure 10 minutes. Results are given in  $\mu M$  of reduced  
ferricyanide/ $mg$  of chlorophyll/hour.

Contraction of swollen chloroplasts upon the addition of ATP to the medium is a mechanochemical processes which can be compared to a similar process in mitochondria (Lehninger, 1962). Such a comparison is corroborated by separating protein possessing the properties of actomyosin from chloroplasts (Ohnishi, 1964). As the contraction of the actomyosin-like protein is effected in proportion to disintegrated ATP, adenosine triphosphatase activity may serve as a measure of intensity of the mechanochemical process. It has been found that the influence of heating the activity of chloroplast ATPase rises approximately threefold.

As a result of heating, free fatty acids (FFA) accumulate in the chloroplasts. A mixture of free fatty acids was separated as referred to in the methodical part. An addition to the suspension of fresh chloroplasts of FFA separated from heated chloroplasts suppresses the Hill reaction (Table 2). Non-heated chloroplasts do not contain FFA to any appreciable amount and an extract from them does not suppress the Hill reaction. The inhibiting action of FFA is inverted to a

Table 2

<u>Additions</u>		<u>% of inhibition</u>
None	I65	
+ FFA from non-heated chloroplasts	I58	4
+ FFA from heated chloroplasts	59	65
None	I58	
+ FFA from heated chloroplasts	5	97
+ FFA from heated chloroplasts+ATP, Mg <sup>++</sup> and SA	78	51

Gas-chromatographic analysis has shown that linolenic acid accounts for 55 molar % in the mixture. Unsaturated fatty acids are strong inhibitors of oxidative phosphorylation in mitochondria (Wojtczak, Lehninger, 1961) and of photochemical reactions in chloroplasts (Krogman, Jagendorf, 1959).

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injuries of chloroplasts. Conformational changes in the actomyosin-like protein of chloroplasts seem to occur under their action, which results in swelling. The likelihood of such an influence of fatty acids on the tertiary structure of protein has been established in model experiments (Mosolov, 1964).

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