REGULATION OF LIGHT-INDUCED SWELLING OF ISOLATED CHLOROPLASTS

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A study on light-induced structural changes in isolated chloroplasts has led to some conclusions and assumptions, which could be summarized as follows: 1. Structural changes are associated with a transfer of electrons (Izawa, Good, 1966); 2. There is a dependence between a high-energy state of chloroplast (X_E) and direction (swelling - contraction) of structural changes (Hind, Jagendorf, 1965);3. Swelling and contraction are connected with the light-induced shift in the internal pH of chloroplast (Deamer,Crofts, Packer, 1967).

The present paper provides some experimental results,which testify a possibility of the key role of ATPase in the changes in structural states of chloroplasts.

Materials and methods. The chloroplasts were isolated from leaves of Vicia faba after Izawa and Good (1966). The crude preparations of chloroplast were resuspended in a medium containing sucrose (0.25M), tris-HCl buffer (0.025 M) pH 7.2 and, after repeated centrifugation, was suspended in the same medium (stock suspension).The swelling and contraction of chloroplasts were followed spectrophotometrically at 520 mμ.

Abbreviations: MA, methyl amine hydrochloride; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; CMB, p-chloromercuribenzoate; PMS, N-methylphenazonium methosulfate; DPS, dimercaptopropane sulphonate, sodium salt.

Results and discussion . The illumination of the suspension of chloroplasts in the presence of ferricyanide causes a decrease in its optical density.Maximum swelling was observed after ca. 20 min (Fig. 1).Swelling was not reversed when illumination was switched off and chloroplasts retained their swollen state in darkness for indefinitely long time. Rate and degree of swelling increase in the presence of uncouplers - MA and NH_4Cl. Hill reaction, as was shown by experiment, is stimulated by MA in 5 times. The inhibition of Hill reaction by DCMU suppresses swelling process completely even in the presence of MA. Chloroplasts exhibit no swelling at illumination in absence of electron acceptors. Addition of ATP and Mg^{++} to a swollen chloroplasts results in their partial contraction. Rate and degree of contraction are not affected by illumination. ATP and Mg^{++} cause not only a contraction of swollen chloroplast,their presence essentially reduces the rate of light-induced swelling. In the presence of uncouplers (MA, NH_4Cl) swelling is not affected by ATP and Mg^{++}.

Analogous results were obtained in a cyclic transfer of electrons,catalysed by PMS.In the presence of PMS swelling proceeds at a very high rate and attains a plateau after 5-7 min. MA and NH_4Cl do not affect the degree and rate of swelling, when PMS is present. Probably a cyclic transfer of electrons provides a complete saturation of swelling, associated with it, and does not require additional activation by uncouplers.

These results testify that the degree of light-induced swelling can be regulated by processes in which ATPase participates. This supposition was confirmed by the experiments where the influence of some ATPase inhibitors on swelling process

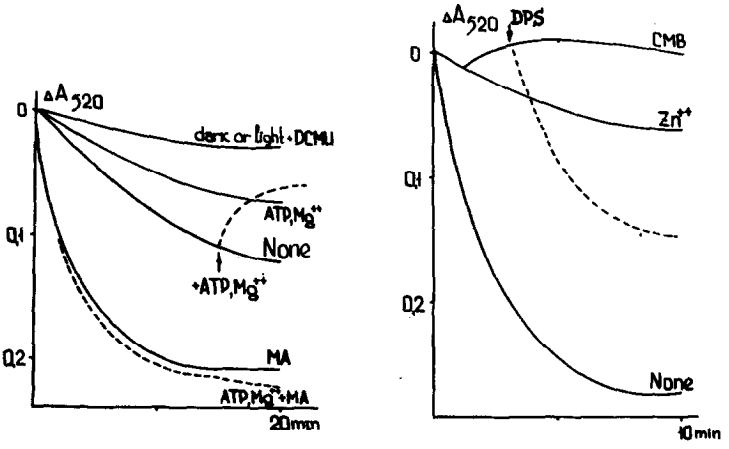

Fig. 1. Optical density changes (ΔA_{520}) of chloroplasts suspension and influence of ATP on it.
The medium contains NaCl (150 mM), tris-HCl buffer pH 7.2 (25 mM), ferricyanide (0.5 mM). Additions, as indicated, to a final concentration of MA (60 mM), DCMU (5 μM), ATP (3 mM), $MgCl_2$ (2.5 mM).Illumination 10,000 lux.

Fig.2. Influence of ATPase inhibitors on optical density changes of chloroplasts suspension.
Experimental conditions as Fig. 1, except that instead of ferricyanide PMS (20 μM) is added.Additions, as indicated, to a final concentration of $ZnCl_2$ (10 mM), CMB (0.1 mM), DPS (1mM).

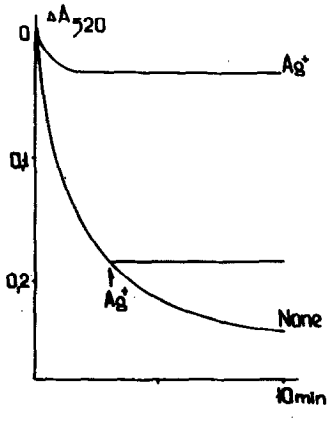

Fig. 3. Influence of Ag^+ on optical density changes of chloroplasts suspension.
The medium contains $NaNO_3$ (150 mM), PMS (20 μM). Addition of $AgNO_3$ to final concentration 0.01 mM.

was investigated (Fig. 2). $ZnCl_2$, suppressing ATPase at 1 mM concentration (Avron, 1962), exhibits an inhibition of swelling only at high concentration (10 mM). Swelling was completely suppressed by CMB, which is a strong inhibitor of ATPase (Wessels, Baltscheffsky, 1960).Swelling was restored again, after the action of CMB, if sodium dimercaptopropane sulphonate was added to a suspension. A strong inhibitor of light-induced swelling of chloroplasts is Ag^+ (Fig. 3).

All above-mentioned reagents (CMB, Zn^{++},Ag^+) can block SH-groups in proteins. A specific function of SH-groups in swelling process is confirmed by the fact, that DPS restores swelling after the effect of CMB (Fig. 2). We have not managed so far to elucidate, whether suppression of light-induced swelling of chloroplasts by CMB, Zn^{++},Ag^+ is associated with their influence on ATPase or on some other proteins, containing SH-groups.

It may be mentioned here, that CMB stimulates Hill reaction by 100%, due to its uncoupling effect. These results prove, that light-induced swelling is only indirectly related to electron transfer, but is intimately connected with processes where SH-containing proteins participate.

The changes in optical density of a suspension reflect the real structural changes in chloroplasts. This was confirmed by the direct determinations of weights of chloroplasts pellets after corresponding expositions.

These experimental results enable us to make following conclusions:

1. Light-induced swelling of chloroplasts of Vicia faba leaves closely associated with the processes coupling with the electron transfer, but not with the transfer itself. The

swelling is stimulated by some uncouplers, those stimulate the flow of electrons (MA, NH_4Cl) and, at the same time, is suppressed some another substances also stimulated this flow (CMB).

2. Swelling could be inhibited or reversed under conditions when high energy bond of ATP realized.

3. ATPase, probably, occupy an important role in light-induced swelling of chloroplasts. Compounds, which undoubtedly block the activity of ATPase, at the same time, suppress swelling.

4. Light-induced swelling of isolated chloroplasts is attributed to a rapid transformation of SH-groups in proteins. Inactivation of SH-groups stops the structural changes in chloroplasts.These SH-groups do not participate in electron transfer (their inactivation with CMB increases Hill reaction).

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