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Proton flow through the ATP synthase in chloroplasts regulates the distribution of light energy between PS I and PS II

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The involvement of ATP synthase in the imbalance between the photoactivities of PS I and PS II under light-limiting conditions, was examined in broken lettuce chloroplasts using modulated fluorimetry. The imbalance, in favor of PS II, was minimal and roughly constant between pH 6.5–7.3 (ratio of PS II/PS I activities about 1.1), and maximal at pH 8.5 (ratio of PS II/PS I activities about 1.4). This increase was strongly inhibited by a treatment of the chloroplasts with the CF_o ATP synthase inhibitor DCCD, but unaffected by the CF_1 ATPase inhibitor, tentoxin. However, tentoxin plus ADP·P_i did inhibit the high pH-induced increased imbalance. These results, when considered with the previous results on the effect of high pH on proton flux through the ATP synthase, suggest that the rate of such proton flow controls the imbalance between the two photosystems. It is possible that there is an in vivo fine-tuning regulating mechanism of the photosystems imbalance via the opening and closing of proton gradient dissipation through the ATP synthase. This mechanism may help alleviate photoinhibitory damage.

Photosystem I; Photosystem II; ATP synthase; Proton flow; Light excitation imbalance; Chlorophyll fluorescence

1. INTRODUCTION

The distribution of light excitation between photosystem I (PS I) and photosystem II (PS II) of oxygenic photosynthesis under light-limiting conditions is regulated by a variety of factors (e.g. light intensity, wavelength, ionic strength). The in vivo state 1 to state 2 transitions is one example of changes in the light distribution which probably involves the redistribution of pigment-protein complexes between PS II and PS I [1–5]. In vitro, there is a marked imbalance in favor of PS II in the photoactivities of PS II and PS I provided the thylakoids are suspended in a medium containing a high level of cations [5,6].

Increasing external pH from 7 to 8.5 was previously shown [6] to increase the imbalance in favor of PS II under light-limiting conditions. A similar pattern of pH dependence was previously observed by Avron [7] for the steady-state rate of electron transport. In a recent study, Evron and Avron [8] have shown that the medium pH-dependent increase in the rate of electron transport was due to an induced leak of protons through the ATP synthase at the higher pH values. This increased proton efflux was strongly inhibited by DCCD, ATP or ADP and P_i under non-phosphorylating conditions. The aim of the present work was to examine whether the increased leakiness of the ATP synthase to protons at the higher pH values may be the cause of the increased imbalance under the same conditions. The evidence suggests an intimate involvement of proton flux through the ATP synthase in regulating the photosystems' imbalance.

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Abbreviations: PS, photosystem; ATP, adenosine tri-phosphate; ADP, adenosine di-phosphate; MeV, methylviologen; DCCD, *N,N'*-dicyclohexyl carbodiimide; TTX, tentoxin

2. MATERIALS AND METHODS

2.1. *Chloroplasts preparation*

Broken chloroplasts from market lettuce were prepared and stored in liquid nitrogen in a concentration of about 3 mg/ml, as previously described [9]. The storage buffer contained 0.3 M sorbitol, 20 mM HEPES, 10 mM NaCl, 5 mM $MgCl_2$ and 30% (v/v) ethyleneglycol, pH 7.3. Total chlorophyll concentration was determined according to [10].

2.2. *DCCD treatment and reaction mixtures*

Chloroplasts (containing 0.5 mg/ml chlorophyll) were preincubated for 30 min in ice and in the dark, in a reaction mixture containing 10 mM HEPES and 10 mM MOPS adjusted to pH 8.0 with NaOH, 10 mM $MgCl_2$, 10 mM NaCl, 200 μM methylviologen (MeV) and 200 μM DCCD in ethanol. Control chloroplasts were preincubated in the absence of DCCD and with ethanol. Final ethanol concentration during the pre-incubation did not exceed 2%.

For fluorescence measurements, chloroplasts were diluted 50-fold in a 2-ml cuvette, so that the concentration of chlorophyll did not exceed 10 μg/ml. The standard reaction mixture contained 10 mM HEPES and 10 mM MOPS adjusted to the desired pH between 6.5 and 9, 10 mM NaCl, 10 mM $MgCl_2$ and 200 μM MeV. Where indicated, 2 mM P_i, 0.1 mM ADP or 1 μM Tentoxin (TTX) were added.

2.3. *Chlorophyll-a fluorescence measurements*

Modulated chlorophyll-*a* fluorescence was measured by a home-built fluorimeter [6]. The exciting modulated light was of short wavelength obtained with a narrow band (10 nm interference filter, peak at 480 nm) with max. intensity of approximately 3.5

$nE \cdot cm^{-2} \cdot s^{-1}$. This intensity was sufficient to exert an actinic effect but still within the light-limiting range of electron transport. The fluorescence signal at 683 nm from the sample was delivered to a photodiode detector and processed by a lock-in amplifier whose output, corresponding to the amplitude of the modulated fluorescence, was recorded on a strip chart recorder. A second non-modulated light source served to exert actinic effects. It was either a broad-band blue light obtained by use of a band filter, serving to saturate electron transport (intensity about 120 $nE \cdot cm^{-2} \cdot s^{-1}$) or alternatively, far-red light (720 nm) serving as light 1 obtained through an interference filter (maximum intensity of about 30 $nE \cdot cm^{-2} \cdot s^{-1}$). Light intensities were varied with neutral-density filters. Before each measurement, the chloroplasts were suspended in the reaction mixture and incubated in the dark for at least 2 min. All measurements were made at room temperature.

Excitation under light-limiting conditions with the weak modulated 480 nm light resulted in a steady-state fluorescence signal, F_s. Superimposing actinic photosynthetically saturating light on the measuring modulated beam yielded a momentary maximal saturated modulated fluorescence signal, F_m, which was also checked occasionally for no further increase by the introduction of DCMU. Superimposing the 720 nm light lowers fluorescence momentarily to a minimum fluorescence level, taken to represent the parameter F_o [11]. Further details are given in [6].

2.4. *Expression of photo-activity distribution between the photosystems*

The ratio of the light activity distribution coefficients of PS II and PS I (β and α resp.) are related to the chlorophyll fluorescence parameters, as

follows [11]: $\alpha \propto F_m - F_o$
$\alpha \propto F_m - F_s$

The distribution of photoactivity between the two photosystems is expressed in terms of $(\beta/\alpha) - 1$, i.e. the relative deviation from full balance between the two photosystems. In this case no assumption with respect to a possible waste of light energy is made (e.g. assuming that $\alpha + \beta = 1$ [6,11].

3. RESULTS

Fig. 1 describes a typical time course of the modulated fluorescence signal in chloroplasts treated with DCCD (a specific inhibitor of proton flow through the ATP synthase, see also [8]), and in control chloroplasts at pH 8.5. The imbalance was twice as high in the untreated than that in the DCCD-treated chloroplasts, indicating a higher photoactivity distribution in favor of PS II in the untreated chloroplasts at pH 8.5. This effect was mostly pronounced at relatively high intensity modulated 480 nm incident light (although still in the light limiting range, (i.e. 2–3 $nE \cdot cm^{-2} \cdot s^{-1}$). The same effect was obtained when either freshly isolated or frozen chloroplasts were used.

Evidently, the light-induced transient in F_s was deeper in the presence of DCCD than in it's absence. The depth of the transient in the presence of DCCD seems similar to that obtained at pH of around 7 in the absence of DCCD [6]. Accordingly, the imbalance was pH-dependent in untreated chloroplasts [6] with the imbalance term, $\beta/\alpha - 1$, increasing from pH 6.5 to pH 9 with a maximum at pH 8.5 (Fig. 2). However, in a similar fashion to the behaviour of electron transport [8], changing the pH with the DCCD-treated chloroplasts had no effect on the imbalance, so that the imbalance remained relatively low throughout the entire pH range studied. Thus, DCCD had a strong effect on the imbalance at pH around 8.5, but essentially very little effect around pH 7.3.

As can be seen in Fig. 3, adding tentoxin (a phosphorylation inhibitor which binds to the CF_1 part

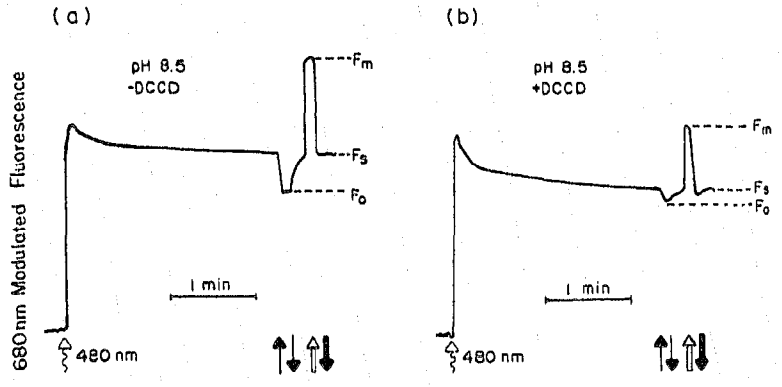

Fig. 1. Modulated 680 nm fluorescence in the absence and presence of DCCD at pH 8.5. Chloroplasts (0.5 chlorophyll mg/ml) were incubated for 30 min in ice and in the dark in the absence (a) or presence (b) of 200 μM DCCD. The incubation buffer contained 10 mM HEPES, 10 mM MOPS adjusted to pH 8.0 with NaOH, 10 mM $MgCl_2$, 10 mM NaCl and 200 μM MeV. For fluorescence measurements, the chloroplasts were suspended at pH 8.5 in a reaction buffer containing 10 mM HEPES, 10 mM MOPS, 10 mM NaCl, 10 mM $MgCl_2$ and 200 μM MeV. Fluorescence parameters F_o, F_s and F_m were measured as described in section 2 with a 480 nm modulated light (approx. 2.2 $nE \cdot cm^{-2} \cdot s^{-1}$), saturating non-modulated broad-band blue light (approx. 120 $nE \cdot cm^{-2} \cdot s^{-1}$) and saturating 720 non-modulated light (approx. 30 $nE \cdot cm^{-2} \cdot s^{-1}$). The upward open arrows denote the turn-on of the saturating light to obtain F_m. The upward closed arrows denote the turn-on of the far-red light to obtain F_o. Closed downward arrows denote the turn-off of either the saturating blue or far-red lights. The wavy arrow denotes the turn-on of the modulated light. Other details are as described in section 2.

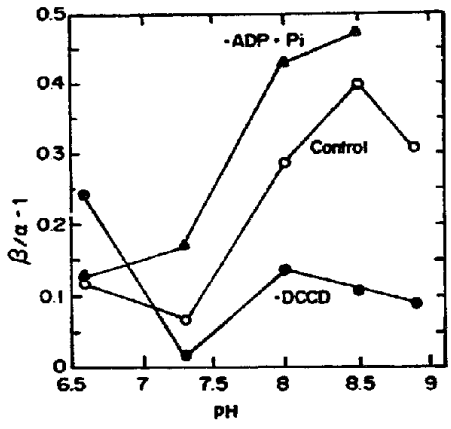

Fig. 2. The effect of DCCD and phosphorylating conditions on the pH-dependence of the deviation from full balance, $\beta/\alpha - 1$. Chloroplasts were pre-incubated in the absence (control, open circles) or in the presence (closed circles) of DCCD. In order to examine the effect of phosphorylating conditions, ADP (0.1 mM) and P_i (2 mM) were included in the reaction buffer (closed triangles). Fluorescence parameters F_o, F_s and F_m were measured at the different pH values as described in section 2. The imbalance term, $\beta/\alpha - 1$ was calculated from the fluorescence parameters as described in section 2.

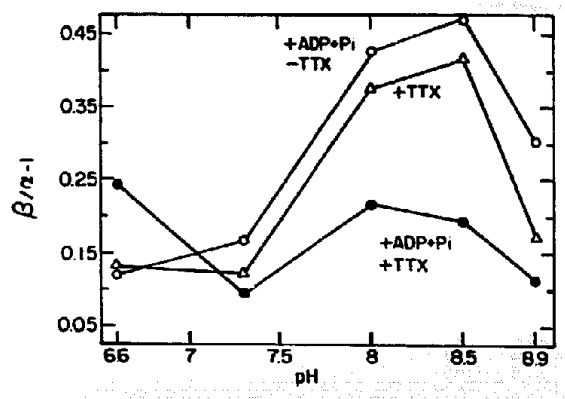

Fig. 3. The effect of tentoxin, ADP and P_i on the pH dependence of the deviation from full balance, $\beta\alpha - 1$. Chloroplasts were suspended in a reaction medium containing 10 mM HEPES, 10 mM MOPS adjusted to different pH values with NaOH, 10 mM NaCl, 10 mM $MgCl_2$ and 20 μM MeV. Tentoxin (1 μM) was added to the reaction mixture in the presence (closed circles) or in the absence (open triangles) of 0.1 mM ADP and 2 mM P_i. A control experiment in the presence of ADP and P_i but in the absence of tentoxin was also performed (open circles). Fluorescence parameters F_o, F_s and F_m were measured at different pH values as described in section 2. The imbalance term, $\beta/\alpha - 1$ was calculated from the fluorescence parameters. Other details are as described in section 2.

of the ATP synthase), instead of DCCD, did not affect the pH dependence of the imbalance. However, the combination of Tentoxin with ADP and P_i affected the imbalance in a manner resembling the DCCD effect. Adding ADP and P_i in the absence of an inhibitor, caused an increase in the imbalance (and phosphorylating proton efflux through the ATP synthase) which was mostly pronounced at pH range between 7 and 8 (Fig. 2). These results are in perfect agreement with the results observed with the same reagents on the pH dependence of electron transport [8].

4. DISCUSSION

The results presented above suggest that proton flow through the ATP synthase can affect the distribution of photoactivity between PS I and PS II. This relationship is of particular interest since the ATP synthase is believed to be located mostly in the grana edges and in the stroma lamellae with no direct contact to PS II which is believed to be located within the grana [12,13]. In a previous study [6] it was concluded that the imbalance is not affected directly by the bulk ΔpH. The results presented here support this suggestion since the marked decrease in the imbalance due to the inhibition of proton flux through the ATP synthase (i.e. by DCCD treatment) is accompanied by only slight changes in ΔpH (around a 0.1 pH unit, not shown).

The results presented in this article are strikingly similar to the results of Evron and Avron [8] in which they showed that high external pH induces proton efflux through the ATP synthase which results in an increased rate of electron transport. It was suggested [8] that this pH-induced proton leak and its closure by the phosphorylating agents may serve as a regulatory valve, eliminating potential damage from an excessive ΔpH and permitting electron flow to proceed under conditions where coupled phosphorylation is blocked.

The results presented here suggest an additional regulatory role to the high pH-induced proton leak through the ATP synthase. When inorganic phosphate availability is limiting ATP synthesis, the proton leak through the ATP synthase will be activated by an unknown mechanism, resulting in a shift of photoactivity in favor of PS II. Under these conditions also CO_2 reduction is impaired and the reducing entities formed in PS I react with oxygen to form deleterious free oxygen radicals [14,15], thus the shift in favor of PS II reduces the potential photo damage due to less light reaching PS I. All the experiments in this study were performed using low light intensities in the light-limiting range. With saturating light intensities, the effect of the proton leakage through the ATP synthase on the maximum rate of electron transport may be even more dramatic than that observed here on the photosystems imbalance [7,8]. Unfortunately the photosystems imbalance cannot be measured under saturating light because of experimental limitations. The mechanism by which proton flux through the ATP synthase affects the imbalance is still unclear.

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