CONTROL OF PHOTOSYNTHETIC OXYGEN EVOLUTION BY THE INTERNAL pH OF THE CHLOROPLAST THYLAKOID

Inhibition of photosynthetic oxygen evolution by uncouplers at high pH and restoration of electron flow by an artificial electron donor for photosystem II

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1. Introduction

Current thinking on the mechanism of energy conservation in photosynthetic electron transport in chloroplasts, triggered by the chemiosmotic hypothesis, proposes vectorial electron flow across the membrane [1,2]. As recently reviewed [3,4] there is, indeed, reasonable convincing evidence to support the idea of oriented photosynthetic electron flow across the thylakoid membrane of chloroplasts, except for contradictory results as to the localization of the oxygen evolution system. A number of experimental data support the notion that the water splitting reaction occurs on the inside of the thylakoid membrane (for example see refs. [2,5–7]), whereas others with chemical probes [8,9] or antibodies [10], indicate an accessibility of the water splitting reaction, i.e. a location on the outside. We wish to report on results which indicate that the water splitting reaction is controlled by the internal rather than external pH and therefore is located towards the inner face of the thylakoid membrane.

2. Results

In the optimal external pH range, electron flow in washed thylakoid preparations (broken chloroplasts) under coupling conditions is slightly stimulated by an uncoupler [11]. As has been observed with different uncouplers before [11–13] at an external pH of 9 and above, the uncouplers gramicidin and nigericin inhibit electron flow from water to an acceptor of photosystem I. If benzidine/ascorbate, an artificial donor system for photosystem II [15], is added, electron flow is restored (fig. 1 and tables 1 and 2). Addition of benzidine/ascorbate to the control does not change the electron flow rate at either pH.

The inhibition by uncouplers at high pH of non cyclic electron flow from water to an acceptor of photosystem I is therefore due to a specific inhibition of the oxygen evolution system. The inhibition of oxygen evolution by gramicidin requires certain time of incubation, as seen in fig. 1. The reversal of the uncoupler inhibition of electron flow by the artificial electron donor is independent of the acceptor used. In table 1 and fig. 1 NADPH formation is measured, whereas in table 2 oxygen uptake via the auto-oxidizable anthraquinone sulfonate is measured.

The restored electron flow rate is sensitive to DCMU (table 1 and fig. 1) which serves as a control, that photosystem II is still operating. If the chloroplasts incubated with an uncoupler are washed in sucrose, the water splitting reaction is active again (table 2), i.e. the oxygen evolution is inhibited and not inactivated by the uncoupler.

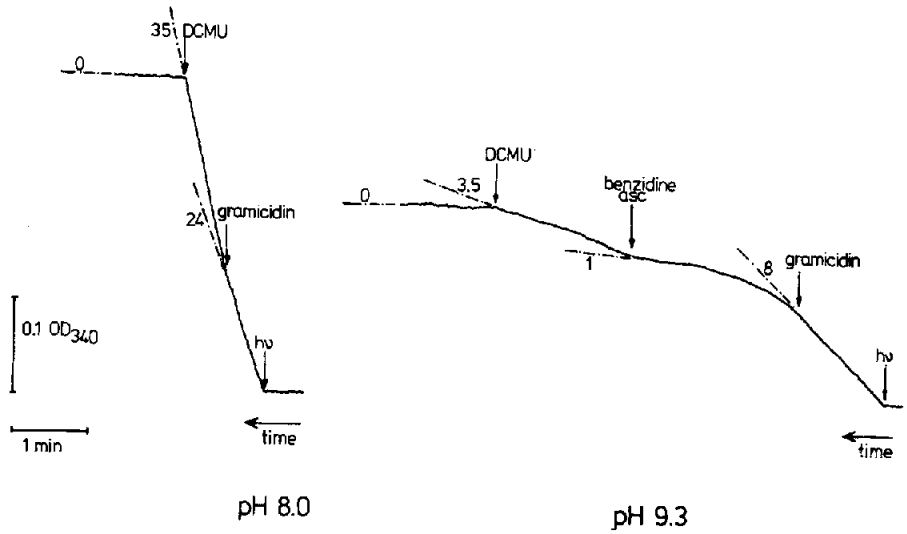

Fig. 1. Reversal by the electron donor benzidine of the inhibition of NADP reduction by 1.5 γ gramicidin at pH 9.3. Assay conditions in table 1. Numbers on the tracing indicate relative rates.

Table 1
Inhibition of photosynthetic NADP reduction at pH 9.0 by an uncoupler and reversal by an artificial electron donor for photosystem II

Additions	μmoles NADPH/hr/mg chlorophyll	
	pH 8.0	pH 9.0
–	66.3	42.5
0.1 γ Gramicidin	66.5	3.6
0.1 γ Gramicidin + 0.5 μmoles benzidine	62	27
1 γ Nigericin	60	0.3
1 γ Nigericin + 0.5 μmoles benzidine	52.5	25.5

Assay conditions: Washed thylakoid preparations from spinach chloroplasts were obtained according to Nelson et al. [14]. Additions in 3 ml in μmoles: 160 tricin/NaOH buffer pH 8.0 or 9.0 respectively; 3 NADP; 0.01 ferredoxin; 10 $MgCl_2$; 5 ADP; 5 P_i; 10 ascorbate and broken chloroplasts with 50 γ chlorophyll; The cuvette was cross illuminated for 3 min with red light of $1.3 \cdot 10^6$ erg/cm² sec at 20°C. The rise in extinction at 340 nm was followed. Gramicidin was obtained from Serva, Heidelberg; nigericin is a gift of Eli Lilly, Ind., USA, obtained through the generosity of Dr. Dilley.

Table 2
Inhibition of pseudocyclic electron flow at pH 9.3 by an uncoupler and reversal by an artificial electron donor for photosystem II

Additions	Electron flow rate μmoles oxygen taken up (15' light)
–	7.2
$2 \cdot 10^{-5}$ M DCMU	1.4
3 γ Nigericin	2.0
3 γ Nigericin + 0.5 μmoles benzidine	5.2
3 γ Nigericin + 0.5 μmoles benzidine + $2 \cdot 10^{-5}$ M DCMU	1.9
–	5.2
1 γ Gramicidin	1.2
1 γ Gramicidin + 0.5 μmoles benzidine	4.1
Chloroplasts incubated for 3 min with 10 γ/ml gramicidin and then washed with 4 M sucrose	3.8

Assay conditions as in table 1 except for pH 9.3 and 0.3 μmoles anthraquinone-sulfonate instead of NADP and ferredoxin. Broken chloroplasts with 100 γ chlorophyll were used. The samples were illuminated with 35 000 lux at 15°C; oxygen uptake was followed manometrically.

3. Discussion

Recent experiments by Rumberg et al. [16–18] and Avron et al. [12,19,20] led to the important conclusion, that it is the inside rather than the outside pH which controls photosynthetic electron flow in the thylakoid membrane. This means that not the pH-optimum of the suspending medium for the chloroplasts but rather the lowered pH inside, brought about by the pH gradient generated upon turning on the light, governs the rate of electron flow. This pH difference outside/inside may be up to 4 units at a pH of 8 in the suspending medium [12]. This conclusion still holds in principal, even though Bamberger et al. [13] observed a more complex dependence of electron flow on the pH inside and outside and on Δ pH.

Avron [12] and Bamberger et al. [13] already pointed out that the known inhibition of electron flow by uncouplers at high outside pH is possibly due to the shifting of the inside pH into the unfavorable pH range maintained outside by the suspending buffer because of the breakdown of the pH gradient. The results of this paper show that the inhibition of overall electron flow by uncouplers at high outside pH is due to a specific inhibition of the water splitting reaction. An artificial donor system for photosystem II like benzidine + ascorbate reverses this inhibition of NADP-reduction by uncouplers by substituting electrons for the inoperative water splitting reaction. The inhibition of oxygen evolution by the uncoupler at high pH is reversible. After removal of the uncoupler the water splitting reaction operates again, also at high outside pH. Therefore it is the optimum of the water splitting reactions, which determines the pH optimum of the overall non cyclic electron flow from water to NADP under these conditions. If the shifting of the internal pH into an unfavorable range, by adding an uncoupler, inhibits specifically the water splitting reaction, it follows that photosynthetic oxygen evolution is located towards the inside of the thylakoid.

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