Correlation between energy-dependent tritium incorporation into CF_1 and light-induced protonation of thylakoid membranes

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Received 23 October 1985; revised version received 13 December 1985

DCCD effects on proton titration of thylakoid membranes and light-induced tritium incorporation in CF_1 were compared. The pool of ionogenic groups exposed in the light (about 0.22 μmol · mg Chl^{-1} in the pH range 7–9) and tritiation of CF_1 were both blocked by DCCD. The results are discussed with reference to the role of the stage of proton (tritium) translocation through the H^+ channel of ATP-synthase in these processes.

Thylakoid membrane *H^+ channel* *ATP-synthase* *^1H-^3H exchange* *Buffer capacity* *(Pea chloroplast)*

1. INTRODUCTION

Energization of thylakoid membranes is known to induce proton-tritium exchange in ATP-synthase. Ryrie and Jagendorf [1] were the first to report that the accessibility of CF_0F_1 polypeptide chains to the exchange with a solvent increased dramatically upon illumination. According to Jagendorf [2,3], parts of the protein are exposed and become tritiated, and are subsequently refolded into regions of the molecule inaccessible to solvent hydrogens. On the other hand there is the well-known fact of light-driven increase in β of chloroplast suspensions in the pH range 7–9, discovered by Polia and Jagendorf [4] and studied by Walz et al. [5]. Opanasenko and Makarov [6] suggested that the light-induced increase of thylakoid membrane buffer capacity was a result of CF_1 ionogenic group protonation.

Abbreviations: Chl, chlorophyll; CF_0 and CF_1, integral membrane portion and catalytic part of the chloroplast ATP-synthase, respectively; DCCD, *N,N'*-dicyclohexylcarbodiimide; NEM, *N*-ethylmaleimide; β, proton buffer capacity; PMS, phenazine methosulphate

In this paper proton titration of thylakoid membranes was used to show that the pool of ionogenic groups exposed in the light (about 0.22 μmol · mg Chl^{-1} in the pH range 7–9) is blocked by DCCD treatment of chloroplasts. Energy-dependent tritium incorporation into ATP-synthase (CF_0F_1) was also investigated. Here we demonstrate that light-induced tritiation is blocked by DCCD. Light-dependent protonation of thylakoids at pH 7–9 and energy-dependent tritium incorporation into CF_1 have been suggested to include the stage of proton (tritium) translocation through the H^+ channel of ATP-synthase.

2. MATERIALS AND METHODS

Chloroplasts were isolated from pea leaves as in [8]. CF_1-depleted chloroplasts were obtained as in [9]. Energy-dependent CF_1 tritiation was carried out according to [1]. The isolation of tritium-treated CF_1 was performed as in [2]. Titration by dilute HCl solution (0.02 M) was assayed in a medium containing 50 mM KCl, 5 mM $MgCl_2$, 50 μM PMS and chloroplasts (0.2 mg Chl · ml^{-1}). Recording and processing of titration curves were

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00145793/86/$3.50

carried out with the help of a laboratory device consisting of an OP-0808 glass electrode (Radelkis, Hungary), an OP-208 precision digital pH-meter (Radelkis, Hungary) connected with a microcomputer (Electronica D3-28, USSR) and titrant dosators. The titration curve in the pH range 9.00–4.00 was recorded in the memory of the microcomputer. The titration data can be presented in a digital form or graphically as a titration curve b(pH) or buffer capacity curve β(pH). The amount of protons bound with thylakoid membranes, H_b^+, was automatically determined as the difference between chloroplast and background titration curves. (The composition of the background solution was the same as in the experiment with the exception of chloroplasts.) Buffer capacity was calculated as the first derivative of sample titration curves:

$$\beta(\mathrm{pH}) = \left| \frac{\mathrm{d}b(\mathrm{pH})}{\mathrm{dpH}} \right| - \beta_m(\mathrm{pH})$$

where β_m(pH) is the background buffer capacity, calculated from the background solution titration curve.

3. RESULTS

3.1. *Identification of ATP-synthase protonation on the buffer capacity curves of thylakoid membranes*

Table 1 shows the results of chloroplast titration by HCl (0.02 M) in the pH range 9–4 in the light and dark. The same data are presented in fig.1 as the dependence of β on pH. As can be seen, chloroplast buffer capacity increases in the pH range 7–9 upon illumination and considerably decreases at pH < 6. The same dependence of the β(pH) light-driven change was found earlier [5]. The titration curve of CF_1-depleted chloroplasts prepared by NaBr (2 M) treatment does not differ from the control (table 1). The results suggest that CF_1 ionogenic groups are likely to make very little if any contribution to the buffer capacity of thylakoid membranes in the dark. The light NaBr-treated chloroplast titration curve is also very close to that of the dark control, i.e. the light-induced increase of proton binding is completely absent at pH 7–9 (table 1).

Fig.1 shows the buffer capacity curves of thylakoid membranes pretreated by DCCD at the concentrations inhibiting photophosphorylation and not affecting the rate of uncoupled electron transport. In the dark β(pH) of DCCD-treated chloroplasts coincides with the control curve. The light-induced buffer capacity peak is not observed on the β(pH) curve of DCCD-treated chloroplasts at pH 7–9, while at pH < 7 the light buffer capacity curve does not differ from that of the light control.

These results suggest that the light-dependent increase of the chloroplast suspension buffer capacity at pH 7–9 is due to protonation of ATP-synthase complex ionogenic groups that cannot be titrated in the dark.

Table 2 shows that other ATP-synthase modifiers and photophosphorylation substrates also affect the amount of ionogenic groups protonated in the light at pH 7–9. (The data are ob-

Table 1

Binding of protons with thylakoid membranes on decrease of pH from 9.0 to 4.0 (H_b^+)

pH	Control		DCCD-treated chloroplasts		NaBr-chloroplasts		DCCD-treated NaBr-chloroplasts	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
9.0⟶8.0	0.33	0.21	0.16	0.18	0.18	0.18	0.17	0.17
9.0⟶7.0	0.72	0.50	0.46	0.51	0.46	0.50	0.46	0.45
9.0⟶6.0	1.18	0.99	0.92	1.01	0.96	1.00	0.95	0.98
9.0⟶5.0	1.75	1.77	1.51	1.78	1.69	1.78	1.61	1.77
9.0⟶4.0	2.83	3.18	2.56	3.20	3.05	3.28	2.64	3.27

The amount of protons bound with membranes was determined as described in section 2. Conditions as in fig.1. Values expressed as μmol H^+/mg chlorophyll

Table 2

Light-dependent protonation of thylakoid membranes in the pH range 7–9: effect of photophosphorylation inhibitors and substrates

	H_b^+ (μmol/mg Chl)	e^- (μmol/h per mg Chl)	Photophosphorylation (% control)
Control	0.22	440	100
+ 10^{-4} M DCCD	0.02	440	0
+ 10^{-6} M $AgNO_3$	0	430	0
NaBr-chloroplasts	−0.04	–	0
+ 10^{-4} M DCCD	−0.01	–	0
+ ADP + P_i	0.08	500	100
+ NEM	0.07	400	30

Photophosphorylation with PMS was measured as in [7]; the rate of the process in the control was 550 μmol/h per mg chlorophyll (100%). The rate of electron transport from water to ferricyanide in the presence of 10^{-6} M gramicidin was measured as in [7]. Chloroplast treatment with *N*-ethylmaleimide was carried out according to [10]

tained as the result of subtraction of the dark titration curve from that in light.) ATP-synthase inhibitors blocking enzyme SH- groups (NEM, $AgNO_3$ and $HgCl_2$) as well as DCCD suppress the light-induced increase of chloroplast buffer capacity at pH 7–9. It should be noted that these inhibitors were used at concentrations such that they did not affect the rate of uncoupling electron transfer from water to $K_3Fe(CN)_6$ and did not change the dark titration curve (not shown). ADP with P_i, as well as ATP, but not ADP alone, decrease the light-induced buffer capacity peak of chloroplast suspensions at pH 7–9.

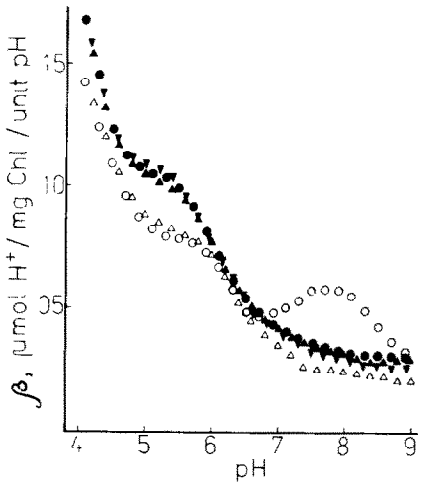

Fig.1. The buffer capacity curves of chloroplasts (○,●), DCCD-treated chloroplasts (△,▲) and NaBr-treated chloroplasts (▼) in the dark (●,▲,▼) and in the light (○,△). The reaction mixture (without chloroplasts) was bubbled to remove dissolved CO_2 at pH 5.0–5.2. Light was turned on when the pH had reached a steady value after the addition of chloroplasts to the reaction mixture (pH 6.0–6.2). The titration was started as soon as the rise in pH due to illumination had stopped. Chloroplast titration both in the dark and light was carried out with 0.02 N HCl in the pH range 9.0–4.0 after alkalinization of the mixture to pH 9.05 with 0.05 N KOH. Preparations were treated with DCCD at 0.5 μmol/mg Chl.

3.2. *Inhibitory analysis of energy-dependent tritium incorporation into CF_1*

Light-dependent tritium incorporation into CF_1 was 100–108 atoms ^3H/molecule CF_1 (table 3). DCCD pretreatment (10^{-4} M, 30 min) of chloroplasts resulted in a decrease of light-driven tritium

Table 3

Effect of ATP-synthase inhibitors on tritium energy-dependent incorporation into CF_1

	Dark	Light	pH 4.5⟶8.5 jump
Chloroplasts	20	110	40
+ DCCD (10^{-4} M, 30 min)	10	10	0
+ NEM[a]	12	32	–

[a] Chloroplasts were treated with NEM according to [10]
Values expressed as atom ^3H/molecule CF_1

incorporation into CF_1 to the level of the dark control. It is essential that CF_1 tritiation induced by a pH jump from pH 4.5 to 8.5 is also completely suppressed by DCCD. Table 3 also shows that tritium incorporation into CF_1 considerably decreases after NEM treatment of chloroplasts.

The obtained results allow one to suggest that the incorporation of slowly exchanging tritium into the soluble part of ATP-synthase (CF_1) is controlled by the process of proton (tritium) transfer through the proton channel of CF_0F_1 complex.

4. DISCUSSION

The analysis of our own and literature data shows that the light-induced pool of ionogenic groups of thylakoid membranes that are protonated in the pH range 7–9 and the pool of CF_1 groups that bind tritium in the light are closely localized systems which are similar in a number of important biochemical parameters: (i) both pools are filled during thylakoid energization. Neither the buffer protonation at pH 7–9 nor tritium incorporation into CF_1 is observed in the dark; (ii) both pools are sensitive to uncouplers [1,2,5]; (iii) proton exchange is completely inhibited by DCCD in both systems (tables 2,3; fig.1); (iv) both the tritium light-dependent incorporation into CF_1 and the increase of buffer capacity at pH 7–9 are decreased due to the effect of ADP with P_i (table 2; [2]); (v) proton (tritium) binding is decreased by the modifiers of SH- groups in both the pools (tables 2 and 3).

Proton capacities of the pools are close and have 170 groups/CF_1 according to the proton titration data (table 2) and 110 groups/CF_1 according to the tritiation results (table 3). However amino acid residues forming the buffer pool probably differ from tightly binding tritium sites. If only ionogenic groups are involved in protonation then NH-groups of peptide bonds, hidden in hydrophobic regions of enzyme molecules most likely take part in a slow ^1H-^3H exchange [11]. At the same time, the obtained results testify that both the processes are similar and likely to be due to a more general phenomenon – proton transfer through the ATP-synthase complex.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Professor L.S. Yaguzhinsky for helpful discussion of this work and Dr A.N. Malyan for his continuous interest and support during the preparation of this paper.

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