Transfer of tightly-bound tritium from the chloroplast membranes to CF_1 is activated by the photophosphorylation process

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Isolated thylakoids were incubated for 14–16 h in the buffer containing 3–6 mCi T_2O/ml and then resedimented and suspended in non-radioactive medium. It was found that illumination of thylakoids induced an increase in radioactivity level in CF_1 isolated from these thylakoids. Such effect was observed only if photophosphorylation substrates (ADP and phosphate or arsenate) were added to the medium during illumination. The light-induced ADP and arsenate-dependent incorporation of tritium into CF_1 was suppressed by DCCD and inhibited by low gramicidin concentrations to the same extent as photophosphorylation.

Thylakoid; Tritium; Phosphorylation; Membrane; CF_1

1. INTRODUCTION

The thylakoid membranes were labelled in the dark with T_2O and re-sedimented in nonradioactive medium, were shown to have a pool of slowly exchangeable tritium [1]. In this paper we describe the photophosphorylation induced migration of this tritium from the membrane into the CF_1 in the labelled thylakoids incubated in the nonradioactive medium.

2. MATERIALS AND METHODS

Thylakoids were isolated from 30–40-day-old leaves of spinach grown under greenhouse conditions according to [2].

Thylakoids labelled with tritium were obtained by 14–16 h incubation in the medium containing 200 mM sucrose, 50 mM NaCl, 2 mM $MgCl_2$, 20 mM sodium ascorbate, 1.5% BSA, 20 mM tricine (pH 8.4), 4 mCi T_2O/ml at 4 mg/ml chlorophyll concentration. Then thylakoids were sedimented and washed from the excess of loosely bound tritium [1] with the medium of the following content: sucrose 200 mM, NaCl 50 mM, $MgCl_2$ 2 mM, tricine 10 mM (pH 8.4). The washed thylakoids were suspended in the standard medium up to a chlorophyll concentration of 0.2 mg/ml. The suspension was supplemented with an electron acceptor (methyl viologen, 10^{-4} M; or PMS, 5×10^{-5} M) and some other components (if necessary) and illuminated with white light for 2 min.

Coupling factor CF_1 was purified according to the method described in [3] with some modification [11]. Fractions containing CF_1 were deionized and separated from T_2O on Sephadex G-25 columns, 12 cm × 0.8 cm, according to [4]. Radioactivity was measured on a liquid scintillation counter Beckman LH-100. The counting efficiency and accuracy were 27% and 2%, respectively. Protein and chlorophyll were assayed according to Bradford [5] and Arnon [6], respectively; photophosphorylation rate was monitored as in [7].

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Abbreviations: CF_1, catalytic part of the chloroplast ATP-synthase; DCCD, *N,N'*-dicyclohexylcarbodiimide; PMS, phenazine methosulphate; MV, methylviologen

3. RESULTS

Table I shows the light-induced incorporation of tritium into CF_1 in tritium-labelled thylakoids washed from unbound radioactivity. It is seen that the amount of label in CF_1 increases by 50% in the light if the photophosphorylation substrates were present. This increase is found to be equal to 8 dpm/μg protein being completely suppressed by DCCD (see also [11]).

It is seen (Table II) that low gramicidin concentrations similarly suppress photophosphorylation and label transfer to CF_1 in the presence of ADP and arsenate.

In the next part of work (Table III) thylakoids after dark incubation for 13 h in the medium containing 5.6 mCi T_2O/ml were re-sedimented and suspended in the standard medium. The suspension radioactivity was 270 μCi T_2O/ml. Then thylakoids suspension was halved and tritium water was added to one portion up to a final level of 1.6 mCi T_2O/ml. As seen from Table III, light-dependent incorporation of tritium into CF_1 in the presence of ADP and arsenate in the both portions was ca 6.5 dpm/μg protein and did not depend on radioactivity of the reaction medium. At the same time, such a dependence was revealed in the absence of photophosphorylation substrates.

The data of Table III indicate that in the absence of ADP and arsenate, tritium incorporation into CF_1 in

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Table I

Tritium incorporation into CF_1 in thylakoids preincubated in the dark for 14 h in the medium containing 3.2 mCi T_2O/ml[a]

Conditions	Tritium label			dpm/μg protein)[b]
	Without inhibitors	DCCD (2×10^{-4} M)	Gramicidin (10^{-9} M)	Gramicidin (10^{-8} M)
Dark	16.0 ± 1.2	15.3 ± 1.2	-	16.0 ± 1.2
Light	16.7 ± 1.3	16.3 ± 1.3	-	16.0 ± 1.2
Dark + ADP, As	18.0 ± 1.4	18.1 ± 1.4	-	17.9 ± 1.4
Light + ADP, As	24.2 ± 2.1	18.3 ± 1.4	18.7 ± 1.4	18.0 ± 1.4

[a]Thylakoids were washed from the main amount of radioactivity and illuminated in the presence of inhibitors (where indicated). CF_1 was obtained from membranes after the removal of the reaction medium, as described in section 2.
[b]The results are means from the 6 experiments. Radioactivity measurement was carried out for 4 min in probes containing 300–400 g of protein in 0.6–1.2 ml. Error, 2% efficiency (27–29%) was controlled by the outer standard. Scintillation liquid volume, 10 ml. Radioactivity of one probe, 1600–3200 cpm

Table II

The effect of gramicidin A on photophosphorylation rate and light-induced incorporation of tritium into CF_1

Conditions	Gramicidin	Photophosphorylation rate	Tritium incorporation into CF_1[a]	
			dpm/μg protein	Δ (light-dark) (dpm/μg protein)
Experiment 1				
Dark	-	-	42.6	-
Dark	10^{-8}	-	42.6	-
Light	-	135	52.4	9.8
Light	5×10^{-10}	56	46.7	4.1
Experiment 2				
Dark	-	-	47.2	-
Dark	10^{-8}	-	47.2	0
Light	-	85	56.6	9.4
Light	10^{-10}	85	52.9	5.7
Light	5×10^{-10}	0	47.9	0.7
Light	10^{-9}	0	47.2	0

Thylakoids were preincubated with T_2O and illuminated after removal of radioactivity from the medium according to [1]. Photophosphorylation rate, mol ATP/mg Chl · h. In Experiment 1 chloroplasts were incubated in the medium of 5.6 mCi T_2O/ml for 17.5 h, whereas in Experiment 2 – in 6.0 mCi T_2O/ml for 19 h.
[a]The probes contained 0.5 mg protein in 0.9–1.2 ml. Radioactivity was measured for 2 min. Error 1% and efficiency 27–29% was controlled by the outer standard. The radioactivity in one probe, 6000–8000 cpm

Table III

Incorporation of tritium into CF_1 at high or low T_2O concentration in the water phase[a]

Conditions	Concentration of T_2O in the medium			
	270 μCi/ml		1.6 mCi/ml	
	Incorporation into CF_1 (dpm/μg protein)	Δ (light-dark) (dpm/μg protein)	Incorporation into CF_1 (dpm/μg protein)	Δ (light-dark) (dpm/μg protein)
Dark	36.2	-	37.4	-
Light	35.2	−1.0	42.5	5.1
Light + ADP, As	42.6	6.4	44.0	6.6

[a]Thylakoids were preincubated for 13 h in the medium of 5.6 mCi T_2O/ml and illuminated after removal of this medium

the light [10] occurs mainly due to transfer of tritium atoms from water phase to ATP-synthase. In the presence of ADP and arsenate, the light-induced incorporation of tritium into CF_1 proceeds by the direct transfer of the label from membrane to ATP-synthase with no transfer through the water phase involved.

4. DISCUSSION

In this work, specific photophosphorylation-dependent transfer of the membrane-bound tritium to CF_1 has been found (Tables I, II).

Tritium bound to membrane proteins is hardly directly involved in the energy coupling. Nevertheless, the following results indicate correlation between the migration of membrane-bound tritium to CF_1 and energy transfer from the electron-transport system to ATP-synthase.

(i) The light-induced increase in transfer of the membrane-bound tritium to CF_1 is observed only under

conditions when the photophosphorylation machinery was functioning, i.e. when ADP and arsenate were present, the process being inhibited by DCCD (Table I).

(ii) Low gramicidin concentrations suppress to the same degree the ATP synthesis and transfer of the membrane-bound tritium to CF_1 (Table II). (For effects of low gramicidin upon photophosphorylation, see [2]).

Two possible interpretations of the above results have been proposed.

(i) The ATP synthesis in the chloroplasts include the proton transfer and the conformational changes of proteins. According to [12,13], these processes do activate the reactions of tritium exchange in the proteins. On the basis of these facts, we suggest that tritium migration during photophosphorylation in tritium-labelled thylakoids resembles the pathway of energy migration in this process.

(ii) According to Skulachev's opinion, the tritium transfer from the membrane to CF_1 is activated by the appearance of the tight mechanical contact between CF_1 and the membrane during the phosphorylation process. Within the framework of this scheme [14], F_1 undergoes phosphorylation-dependent reversible motion in the direction perpendicular to the membrane plane. F_1 is assumed to protrude from the membrane to find its hydrophilic substrates, ADP and P_i, and then contacts the membrane to be affected by transmembrane H^+ potential.

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