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Influence of thylakoid protein phosphorylation on photosystem I photochemistry

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The influence of the phosphorylation of thylakoid proteins on photosystem I photochemistry has been measured under conditions of linear dependence of the rate of electron transport to NADP on light intensity. It was found that the phosphorylation by ATP of light harvesting chlorophyll protein complex (LHC) II and other polypeptides stimulates the rate up to ~ 40%; the stimulation is larger when the wavelength of actinic light corresponds to the main absorption of LHC II.

Photosystem I; LHC phosphorylation; Energy partition; Thylakoid

1. INTRODUCTION

The phosphorylation of LHC II by a thylakoid bound protein kinase and its dephosphorylation by an intrinsic phosphatase is thought to be a mechanism for the regulation of excitation energy distribution among the two photochemical reactions of photosynthesis (reviews [1–3]). The kinase becomes activated when the plastoquinone pool is reduced, and is inactivated when PQH_2 is reoxidized [4]. No regulatory oscillation of activity has been reported for the phosphatase. When PS II is over-excited, the kinase is therefore activated and LHC II is phosphorylated. As a consequence, a fraction of it is removed from the PS II–LHC II matrix (probably due to electrostatic repulsion) and migrates to PS I [5,6]. The result is a decreased excitation of PS II, monitored by a decrease of its fluorescence of ~10–25% [1,2,3,7] as part of the energy is transferred to PS I (which quenches the fluorescence). The enhanced photochemical activity of PS I reoxidizes PQH_2, and this leads to deactivation of the kinase. The activity of the phosphatase will then dephosphorylate LHC II, which migrates again to PS II.

This scheme represents a perhaps somewhat oversimplified model for the regulation of excitation energy distribution, and is applicable only in the presence of cations, when the thylakoids are fully appressed and the transfer of excitation energy from the PS II–LHC II matrix to PS I (the 'spillover') is thought to be largely prohibited by the segregation of the two photosystems from one another in different regions of the membranes [8]. A possible interaction of the effects of cations and protein phosphorylation in the regulation of energy distribution has been recently discussed [9].

While a large body of evidence is available on the decline of PS II fluorescence (and therefore of the energy available to PS II) upon LHC II phosphorylation, the reports on the expected corresponding increase of PS I photochemistry are more contradictory and limited. Farchaus et al.

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Abbreviations: LHC II, light harvesting chlorophyll protein complex II; PS I, PS II, photosystems I and II; PQ, PQH_2, plastoquinone, respectively, oxidized and reduced; P_{700}, the reaction centre chlorophyll of PS I; PMS, phenazinemethosulfate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine

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[10] reported the inhibition of PS II and stimulation of PS I photochemistry by LHC II phosphorylation; however, the interpretation of the results was complicated by an inhibition caused by the preillumination of the thylakoids designed to activate the kinase. Telfer et al. [11] reported the increase at low light intensity of the extent of flash-induced oxidation of P_{700} due to membrane protein phosphorylation. Similar results, though of smaller size, were more recently obtained with PS I enriched vesicles [12] prepared from phosphorylated thylakoids as compared to non-phosphorylated. However, opposite results were also reported [13–15]. The stimulation of PS I electron transport to methylviologen has been reported [16].

Previous reports from this laboratory have shown that steady state PMS-catalyzed cyclic photophosphorylation (an activity dependent upon PS I photochemistry) is stimulated by thylakoid protein phosphorylation whilst the electron transport from water to NADP is not usually affected [17,18]. These latter observations have been explained quantitatively on the basis of opposed effects on the photochemical activities of PS I and PS II of the protein phosphorylation-induced detachment of part of LHC II from PS II and its association with PS I [18].

We report here the stimulation of the steady state rate of electron transport from a PS I donor system to NADP, in the presence of DCMU, measured under conditions where the rate is linearly dependent upon light intensity. The enhancement is larger with actinic light of wavelengths where LHC II absorbs maximally, whilst no enhancement was observed at 725 nm, where LHC II does not contribute to absorption.

2. MATERIALS AND METHODS

Stroma-free thylakoids were prepared as described [17] from spinach leaves collected and kept 1–2 h in darkness. The thylakoids were extracted and constantly kept in the stacked condition, in the presence of 5 mM $MgCl_2$.

The phosphorylation of LHC II (and other membrane proteins) was performed in the dark, as described [17], in the presence of the NADPH-ferredoxin reducing system and 10 mM NaF. After 15 min of phosphorylation in the presence of 1 mM ATP (ATP was omitted in the control), the membranes were diluted 50-fold with ice-cold buffer containing 0.2 M sucrose, 30 mM tricine-NaOH, pH 8.0, 5 mM $MgCl_2$, 10 mM NaF.

The same buffer served as the reaction medium in the measurement of NADP reduction, with the following additions (final concentrations): NADP 0.5 mM, ADP 0.5 mM, P_i 2.5 mM, DCMU 5 μM, ascorbate 5 mM, TMPD 100 μM and ferredoxin at saturating concentration, which was established in different experiments around 3.5–5 μM (depending upon the light intensity). NADP reduction was measured at 20–21°C as the change in absorbance at 340 minus 390 nm in a dual wavelength spectrophotometer (Sigma ZWS, Munich) equipped with side illumination. Actinic light was filtered through 5 cm of water, a heat filter and interference filters of 10 nm half-band width as indicated. Chlorophyll concentration was 10–12 μg/ml. Fluorescence was measured at 682 nm in the presence of 10 μM DCMU, and excited at 475 nm. Chlorophyll was estimated according to Arnon [20].

3. RESULTS

The activity of PS I was measured, under steady-state illumination with light intensities such that the rate of electron transport from $TMPDH_2$ to NADP was linearly dependent upon incident radiation intensity (fig.1). The enhancement of the rate upon LHC II phosphorylation, measured as the slope of the straight lines, was 18.3, 32.1, 24.7 and 25.9% respectively, at 450, 480, 500 and 650 nm. No enhancement was observed at 725 nm, a wavelength where LHCP does not contribute to absorption [20]. A number of such experiments have been carried out with different preparations

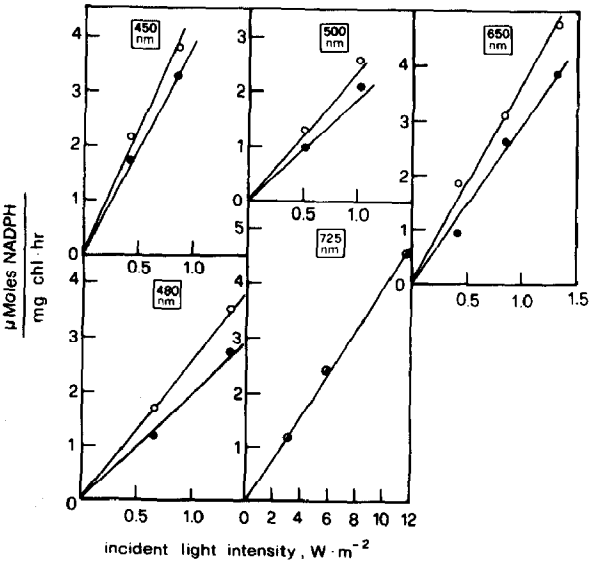

Fig.1. Influence of thylakoid protein phosphorylation on PS I dependent NADP reduction. Conditions as under section 2. Black dots, control; white dot, phosphorylated thylakoids.

Table 1

Effect of thylakoid protein phosphorylation on the rate of PS I-dependent NADP reduction

Actinic light wavelength (nm)	Enhancement of NADP reduction by phosphorylation (%)	
450	23.2 ± 2.6	(4)
480	37.2 ± 4.6	(7)
500	30.0 ± 5.5	(6)
650	27.1 ± 1.0	(3)
671	11.7	(1)
725	00.0	(2)

Conditions: see section 2. Fluorescence decrease due to phosphorylation was 16.9% ± 1.4 (7 preparations). The standard error is indicated, and the number of different preparations is in parentheses

over a period of 4 months. The results obtained are summarized in table 1.

A slight increase of absorbance of 5–7% has been observed upon thylakoid protein phosphorylation (Garlaschi, F.M., personal communication), along most of the absorption spectrum. However, the magnitude of this effect is inadequate to account for the stimulation of electron transport reduced here.

4. DISCUSSION

The observations reported here demonstrate that PS I-dependent NADP reduction is enhanced by the phosphorylation of the thylakoids polypeptide(s), mostly LHC II, under conditions where light intensity is strictly limiting the rate of electron transport. The enhancement of PS I activity is concomitant with the decrease of PS II fluorescence and its extent is dependent on the wavelength of actinic radiation in such a way as to be interpretable in terms of the increase of LHC II-absorbed light available to PS I. The results can therefore be interpreted to support the idea that upon phosphorylation a fraction of LHC II is incorporated into the antenna of PS I. This conclusion is in agreement with the results and the opinion of others [1,2,5,6,9–12]. The experimental evidence presented here relies on the rate of reduction of the physiological acceptor NADP by PS I and on the spectrum of the stimulation effect. It was previously shown that when the complete electron transport chain from H_2O to NADP is in action, no stimulation of NADP reduction by LHC II phosphorylation is usually observed [17,18], owing to the opposed effects of phosphorylation on the two photosystems [18]. Lack of stimulation, but rather inhibition of whole electron transport chain from H_2O to methylviologen has been reported [21]. It is an open question whether the phosphorylation of LHC II in vivo can stimulate directly, under the appropriate conditions, non-cyclic electron transport. The possibility cannot be discarded that in vivo the regulation of photosynthesis by the phosphorylation of LHC II and other membrane proteins involves the function of PS I in both cyclic and non-cyclic electron transport and ATP generation.

This possibility is also suggested by Horton [22], who found that enhancement of O_2 evolution by PS I absorbed radiation is minimal when no ATP is required (nitrite reduction), and maximal when ATP is required (CO_2 assimilation). He has also shown that LHC II phosphorylation is stimulated upon imposing to chloroplasts an ATP deficit.

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