REGULATION OF PHOSPHORYLATION OF CHLOROPLAST MEMBRANE POLYPEPTIDES BY THE REDOX STATE OF PLASTOQUINONE

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1. Introduction

Higher plant chloroplasts possess a light-activated protein kinase that catalyses phosphorylation of several thylakoid proteins including LHCP [1–5]. A decrease in the yield of chlorophyll fluorescence from PSII at room temperature was caused by addition of ATP under conditions necessary for kinase activity. Under the same conditions there is an increase in the relative fluorescence emission from PSI at −196°C. These observations support the proposal that phosphorylation of LHCP controls the distribution of quanta between PSII and PSI [6,7]. On the basis of the ability of various partial reactions of photosynthetic electron transport to promote kinase activity, it was proposed that the redox state of plastoquinone controls protein phosphorylation and hence also the distribution of quanta [8]. A similar suggestion was made to explain ATP-induced fluorescence quenching [7] and potentiometric redox titration indeed showed the involvement of a 2 electron carrier with $E_{m,7.8} \sim 0$–+50 mV [9]. However, it remained to be shown that:

(i) Phosphorylation was responsible for the fluorescence changes,

(ii) Plastoquinone in pea chloroplasts titrates with this midpoint potential.

Here, the crucial links are established between the redox state of plastoquinone, the activation of protein kinase and changes in chlorophyll fluorescence. A model by which the redox state of plastoquinone can control the relative rates of excitation of PSII and PSI is presented.

Abbreviations: PSII, photosystem II; PSI, photosystem I; LHCP, light-harvesting chlorophyll protein; SDS, sodium dodecyl sulphate; M_r, relative molecular mass

2. Materials and methods

Pea chloroplasts were isolated and incubated at defined redox potentials as in [9] except that the chlorophyll concentration was increased to 25 μg/ml and 3 μCi [γ-^{32}P]ATP/ml was present giving 0.15 mM ATP final conc. After 10 min incubation in darkness, samples were withdrawn from the redox cuvette by syringe and precipitated with trichloracetic acid. The proteins were washed with acetone and analysed by SDS–polyacrylamide gel electrophoresis, and the gels stained, dried and autoradiographed, as in [1,4]. Radioactivity incorporated into the LHCP doublet was determined by excising the stained bands from gels and determining radioactivity by Cerenkov counting [1]. After removal of the samples, the F_m levels were recorded. In separate experiments, the decrease in area above fluorescence induction curves (normalised to F_v) was determined after incubation at different redox potentials. Induction curves were recorded and analysed using the procedures in [7,10] except that DCMU was omitted and the time base lengthened to enable F_m to be reached. Under these conditions the area is determined by the size of the acceptor pool (plastoquinone) available to PSII.

High specific activity [γ-^{32}P]ATP was obtained from the Radiochemical Centre (Amersham, Bucks.) and X-ray sensitive film (Kodirex) was supplied by Kodak Limited (Hemel Hempstead, Herts.).

3. Results and discussion

In fig.1, the extent of ATP-induced fluorescence decrease is plotted against the amount of incorporation of ^{32}P-radioactivity into LHCP during a series of

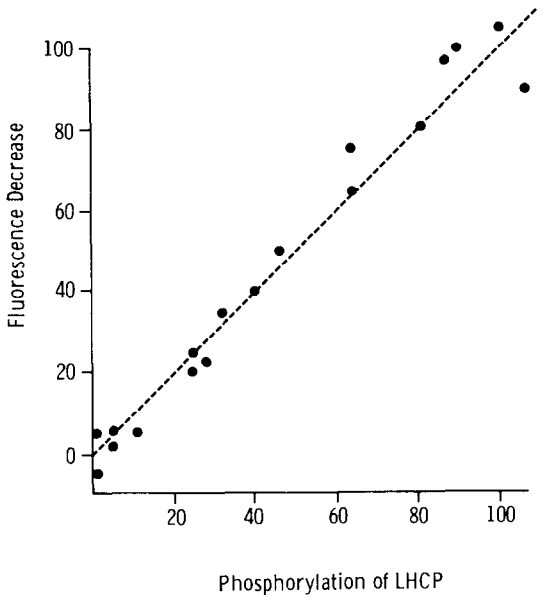

Fig.1. Comparison of ATP-induced fluorescence lowering with the amount of ^{32}P-incorporation into LHCP. Chloroplasts were incubated in darkness at defined redox potentials and the F_m and radioactivity assayed as in the text. The ΔF refers to the decrease in fluorescence compared to a control incubated without ATP. Both ΔF and ^{32}P incorporation are normalised to 100% maximum extent.

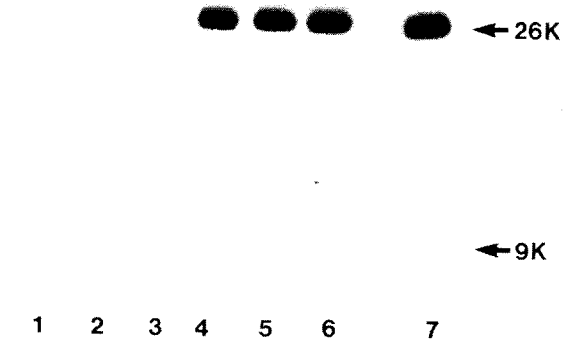

Fig.2. Autoradiograph of chloroplast proteins. Chloroplasts were incubated in darkness with [γ-^{32}P]ATP at defined redox potentials before proteins were extracted and electrophoresed as in the text. Incubation E_h-values were +35 mV (track 1), +75 mV (track 2), +115 mV (track 3), −35 mV (track 4), −100 mV (track 5) and −75 mV (track 6). Track 7 represents a sample incubated in continuous light.

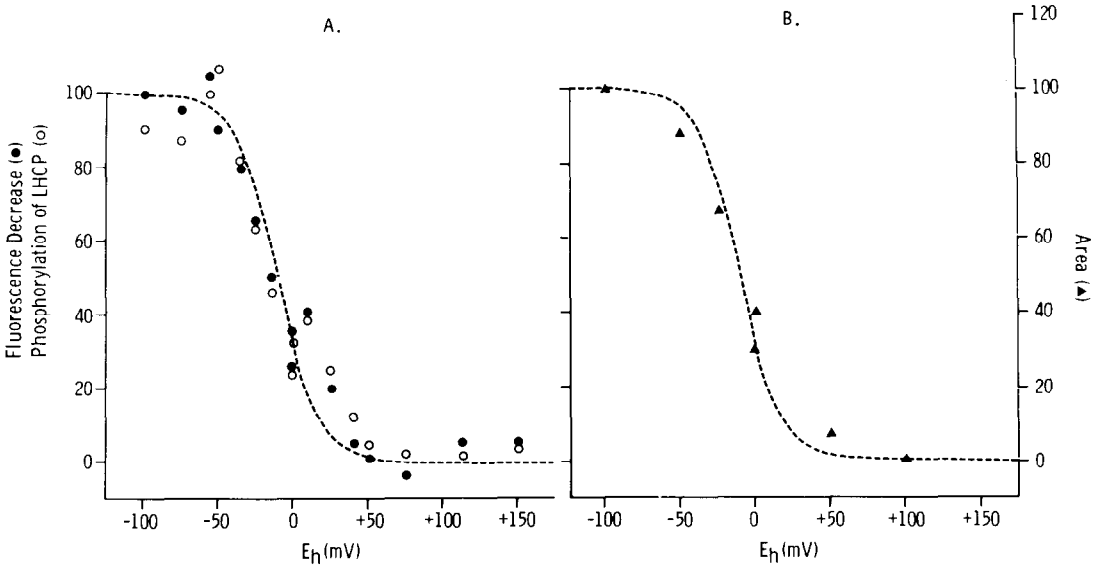

Fig.3. Redox titration of ATP-induced fluorescence lowering, ^{32}P incorporation into LHCP (A) and the decrease in area above the fluorescence induction curve (B). Conditions and assay were as in fig.1 and the text. In (B) the area decrease is normalised to 100%, the maximum decrease over this potential range.

incubations at different redox potentials. The almost perfect correlation between these parameters over a wide range of activity represents the strongest evidence yet obtained that phosphorylation of LHCP is responsible for a decrease in the chlorophyll fluorescence yield in chloroplast membranes.

Fig.2 shows an autoradiograph of chloroplast proteins obtained from samples incubated at different E_h. Track 1 (E_h = +35 mV) shows a small amount of phosphorylation of a 26 000 M_r polypeptide which has been identified as a constituent of LHCP [3]. Under more oxidizing conditions (tracks 2,3) no detectable labelling was seen. In contrast, at more negative E_h (tracks 4–6) considerable phosphorylation of LHCP was observed with the level of phosphorylation then approaching that seen after incubation in saturating continuous light [9] for 10 min (track 7).

In fig.3A are shown the labelling and fluorescence data plotted as a function of the incubation E_h. Both sets of data seem to fit a Nernst equation with $E_{m,\,7.8}$ ~0 mV. The dotted line is an n = 2 plot but analysis of the log ox/red plots shows that the data best fit an n = 1.7 slope. It is not unusual in redox titrations of membrane-associated factors to find n-values which are not whole numbers, probably because of less than perfect redox equilibration. The important conclusion from fig.3A, however, is that a single-equivalent carrier is not involved and most likely the 'true' n-value is 2. Apart from the newly-identified PSI associated carrier which is involved in cyclic electron transport [11], the only n = 2 electron-transport chain component with $E_{m,7.8}$ ~0 mV is plastoquinone [12]. Fig.3B shows a redox titration of the acceptor pool available to PSII, determined under the same conditions as for the data in fig.3A. The acceptor pool is the pool of oxidised plastoquinone [13] and fig.3 shows that it titrates with the same E_m- and n-value as those obtained for activation of protein kinase.

The control of the activity of protein kinase by the redox state of plastoquinone provides the chloroplast with a mechanism by which the relative rate of excitation of PSII and PSI can be regulated (fig.4). Low temperature data [6–9] suggest that the excitation lost from PSII is at least partially transferred to PSI. Thus there exists coherent mechanism by which relative rates of excitation of PSII and PSI can be controlled by the relative rates of electron transfer into (from PSII) and out of it to the plastoquinone pool (PSI). This kind of 'feedback' control can therefore explain the long-established state I/state II transitions

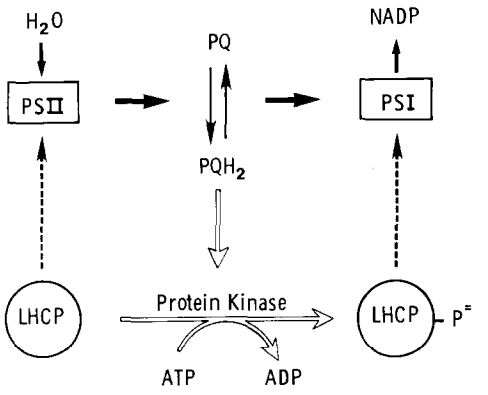

Fig.4. Regulation of distribution of quanta between PSII and PSI by phosphorylation the LHCP. Solid arrows represents the non-cyclic electron-transfer pathway. Broken arrows represent the proposed relative increase in energy transfer to PSI in the phosphorylated state and to PSII in the dephosphorylated state.

[14–16]. As discussed in [7,9,17] phosphorylation of LHCP may also have importance both in preventing over-excitation of PSII under conditions of inhibition of non-cyclic flow and in stimulating cyclic electron transport. This demonstration of the involvement of plastoquinone is entirely consistant with that proposal.

In the course of these experiments it was observed that the other major proteins phosphorylated by a thylakoid protein kinase were labelled when plastoquinone was reduced (see fig.1). Although the data does not allow the fitting of a redox titration curve (because of much lower protein concentration and hence radioactivity) it seems that phosphorylation of these proteins is also dependent on plastoquinone. The fact that one of these has been identified as a CF_0 protein [5] suggests that further study of possible control of photophosphorylation is warranted.

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