Photosynthesis and phosphorylation of light-harvesting chlorophyll *a/b*–protein in intact chloroplasts

Effects of uncouplers

J.F. Allen

Department of Plant Sciences, Baines Wing, The University of Leeds, Leeds LS2 9JT, England

Received 19 September 1983; revised version received 14 November 1983

Protein phosphorylation in isolated, intact pea chloroplasts was measured during the onset of CO_2-dependent O_2 evolution. Total incorporation of ^{32}P (from $^{32}P_i$) into light-harvesting chlorophyll *a/b*–protein was found to be less sensitive than O_2 evolution to inhibition by the uncouplers FCCP and NH_4Cl. It is concluded that changes in the rate of ATP synthesis cannot affect protein phosphorylation without also affecting the rate of CO_2-fixation in this system. The ATP/ADP ratio is therefore unlikely to regulate photosynthetic protein phosphorylation under normal physiological conditions.

Photosynthesis *Protein phosphorylation* *Uncoupler* *CO_2-fixation* *Electron transport* *Chloroplast*

1. INTRODUCTION

Reversible phosphorylation of light-harvesting chlorophyll *a/b*–protein (LHCP) is thought to play a regulatory role in plant photosynthesis. Control of the protein kinase reaction by redox state of plastoquinone seems to be a component of the mechanism which corrects any imbalance in excitation energy distribution between the two photosystems [1,2]. Thus transition to light-state 2 is brought about by reduction of plastoquinone and by phosphorylation of LHCP, while transition to light-state 1 is brought about by plastoquinone oxidation and by dephosphorylation of LHCP.

This hypothesis now enjoys a degree of experimental support (review [3–8]) with redox control of the protein kinase having received particular attention [1,2,9–11]. It has nevertheless been suggested [12,13] that plastoquinone redox state has little effect on LHCP phosphorylation or on excitation energy distribution under normal circumstances, serving merely to inactivate wasteful ATP hydrolysis by the protein kinase in darkness [12]. A further suggestion [12,13] is that physiological control of protein kinase activity is exerted primarily either by relative adenine nucleotide concentrations, by cAMP, or by Mg^{2+} concentration.

How any of these factors might be responsible for state 1–state 2 transitions remains unclear [13]. However, the possibility remains that ATP and ADP regulate thylakoid protein kinase activity in vivo. If this is the case then ATP and ADP concentrations should regulate protein kinase activity without directly inhibiting overall photosynthetic rate – no net gain in efficiency could otherwise result.

This letter describes experiments in which both CO_2-dependent O_2 evolution and phosphorylation of LHCP in intact pea chloroplasts were titrated with the uncouplers FCCP and NH_4Cl. Uncouplers at low concentrations should decrease the steady-state ATP/ADP ratio, and should inhibit LHCP phosphorylation but not photosynthetic rate if the LHCP phosphorylation reaction is primarily under adenosine phosphate control.

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

2. EXPERIMENTAL

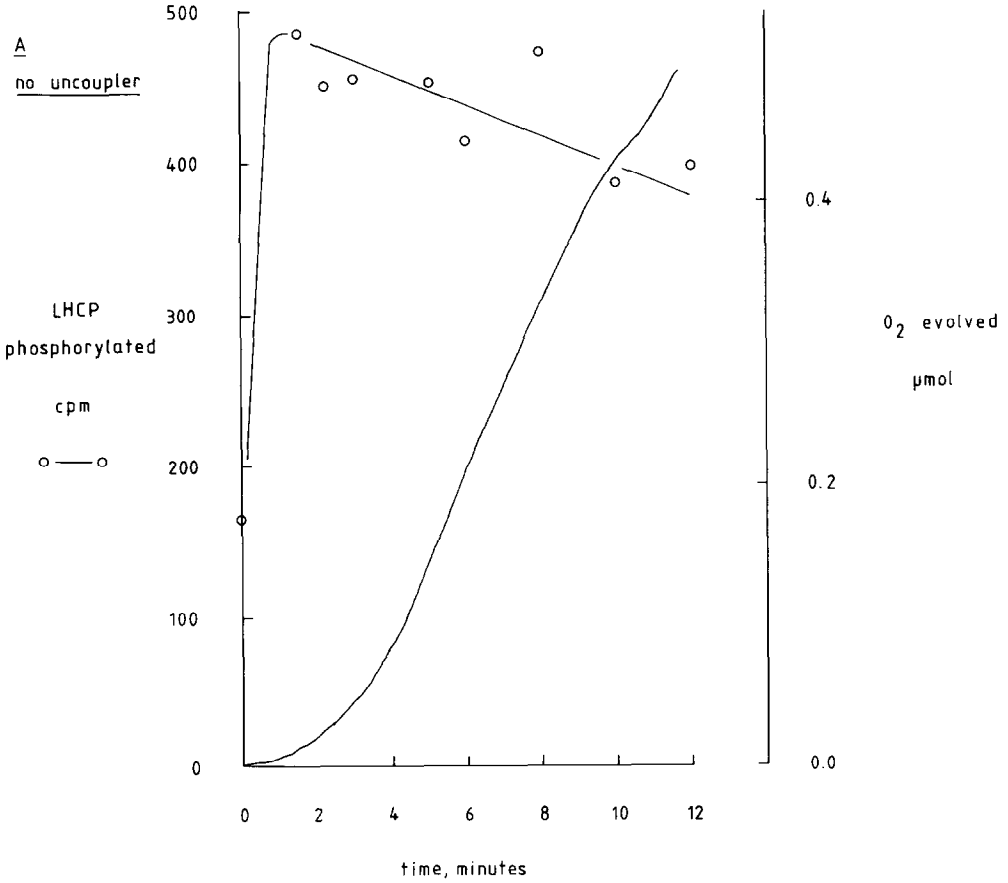
Intact chloroplasts were isolated from shoots of 10-day-old peas as in [14], except that an isotonic sorbitol medium was used both for homogenization and for resuspension of the chloroplast pellet. This medium contained sorbitol (0.33 M), EDTA (2 mM), $MgCl_2$ (5 mM), NaCl (5 mM), ascorbate (10 mM) and Hepes (50 mM) at pH 7.6. Reactions were carried out in the vessel of a Hansatech O_2 electrode which was illuminated by a Volpi 250 W light source via twin fibre-optic bundles and a red filter (600 nm cut-off). The reaction medium (pH 7.6) contained sorbitol (0.33 M), EDTA (2 mM), $MgCl_2$ (1 mM), $MnCl_2$ (1 mM), Hepes (50 mM), $NaHCO_3$ (10 mM), ascorbate (2 mM), $Na_4P_2O_7$ (5 mM), ATP (1 mM), catalase (2500 units), $^{32}P_i$ (500 μCi) and chloroplasts equivalent to 100 μg chlorophyll in a total volume of 1.0 ml. Time-courses were started by switching on the light. At intervals samples (50 μl) were withdrawn and immediately mixed with 5% trichloroacetic acid (1 ml).

Samples were centrifuged and the pellets resuspended directly into buffer for loading on SDS-polyacrylamide gels [14]. After electrophoresis on 10–30% linear acrylamide gradient gels, labelled bands were identified by autoradiography. The prominent LHCP band of each sample was then excised from the gel and its activity measured by Cerenkov counting.

ATP, bovine catalase and FCCP were from Sigma. Carrier-free $^{32}P_i$ was from Amersham.

3. RESULTS

Fig.1A shows a time-course for CO_2-dependent O_2 evolution and for associated changes in the total extent of incorporation of ^{32}P into LHCP. As previously reported [14], LHCP phosphorylation was rapid during the first few minutes of illumina-



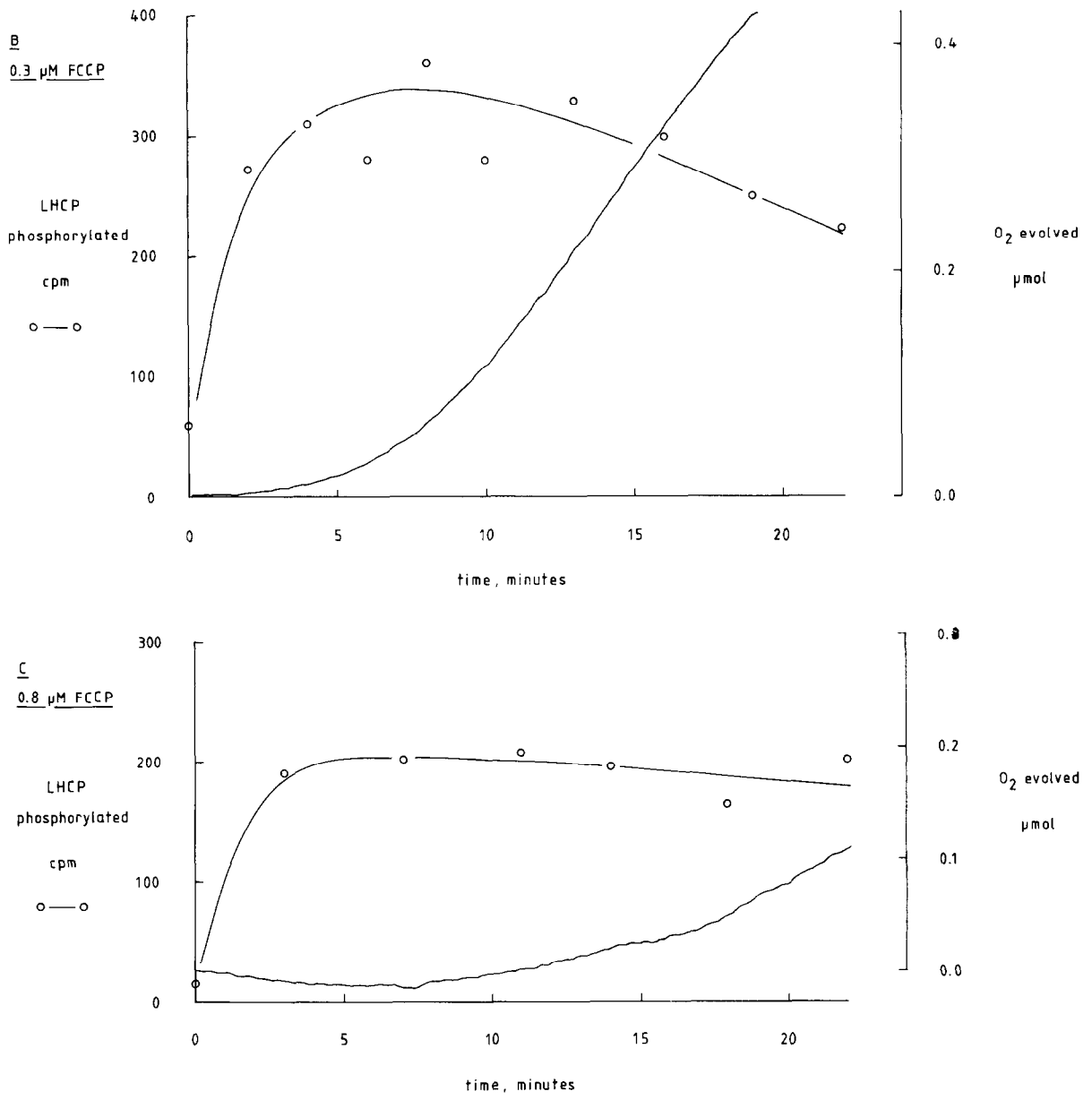
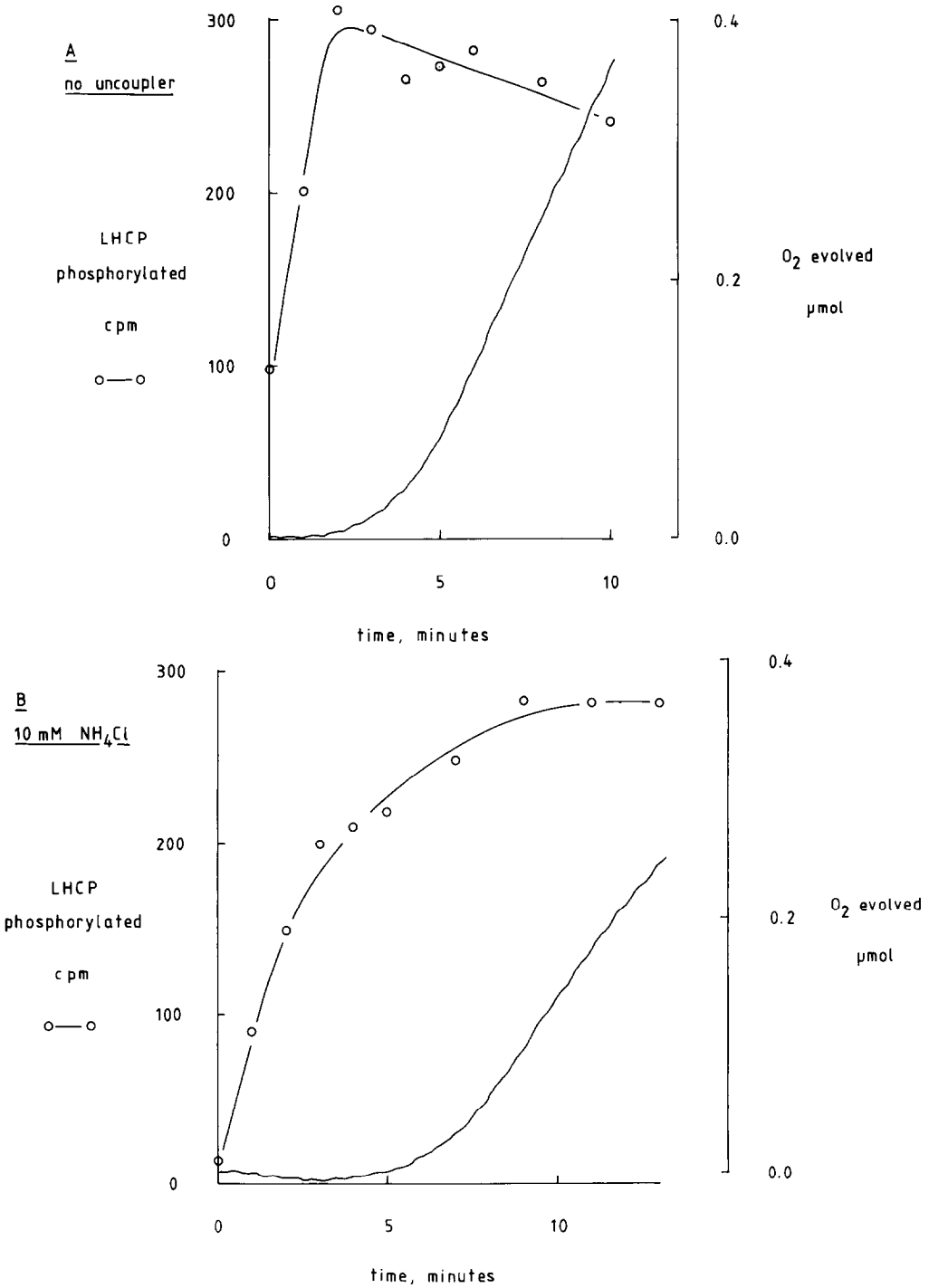


Fig.1. Time-course of CO_2-dependent O_2 evolution and of ^{32}P-labelling of LHCP in intact pea chloroplasts: effects of FCCP. FCCP concentrations and linear rates of CO_2-dependent O_2 evolution are as follows: (A) no uncoupler, 41 μmol·mg Chl^{-1}; (B) 0.3 μM FCCP, 22 μmol·mg $Chl^{-1}\cdot h^{-1}$; (C) 0.8 μM FCCP, 9 μmol·mg $Chl^{-1}\cdot h^{-1}$.

tion, when CO_2-dependent O_2 evolution was slow. The onset of CO_2-dependent O_2 evolution was accompanied by a fall in the level of LHCP phosphorylation, which could in principle be a result either of net oxidation of plastoquinone or of a decrease in the ATP/ADP ratio. Fig.1B shows the corresponding time-courses obtained in the presence of 0.3 μM FCCP, a concentration of uncoupler which extended the lag-phase and which caused 46% inhibition of the rate of CO_2-dependent O_2 evolution. The initial rate of incorporation of ^{32}P into LHCP may also have

decreased, though the maximum level of ^{32}P-incorporation was, however, decreased by about 33%. Fig.1C shows that 0.8 μM FCCP considerably extended the lag-phase of CO_2-dependent O_2 evolution and caused 80% inhibition of the final rate: it also gave about 60% inhibition of the level of ^{32}P-incorporation into LHCP.

Fig.2 shows similar effects of NH_4Cl. Fig.2A



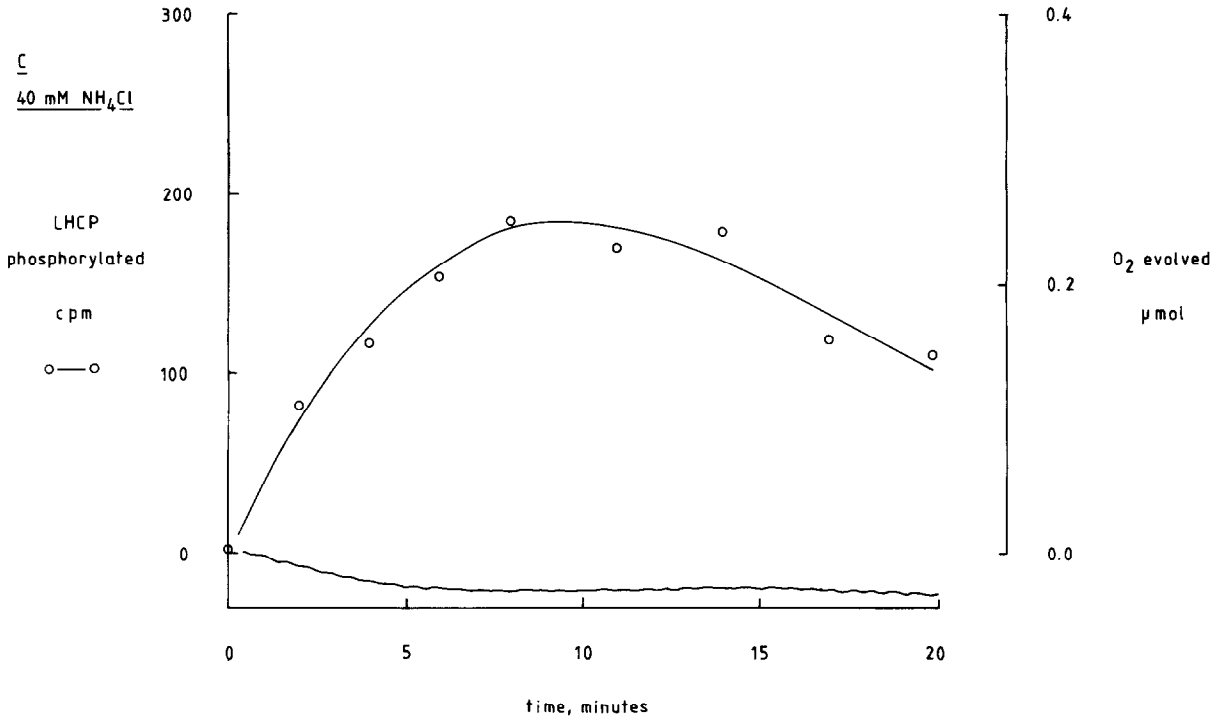


Fig.2. Time-course of CO_2-dependent O_2 evolution and of ^{32}P-labelling of LHCP in intact pea chloroplasts: effects of NH_4Cl. NH_4Cl concentrations and linear rates of CO_2-dependent O_2 evolution are as follows: (A) no uncoupler, 36 μmol·mg $Chl^{-1}\cdot h^{-1}$; (B) 10 mM NH_4Cl, 25 μmol·mg $Chl^{-1}\cdot h^{-1}$; (C) 40 mM NH_4Cl, <1 μmol·mg $Chl^{-1}\cdot h^{-1}$.

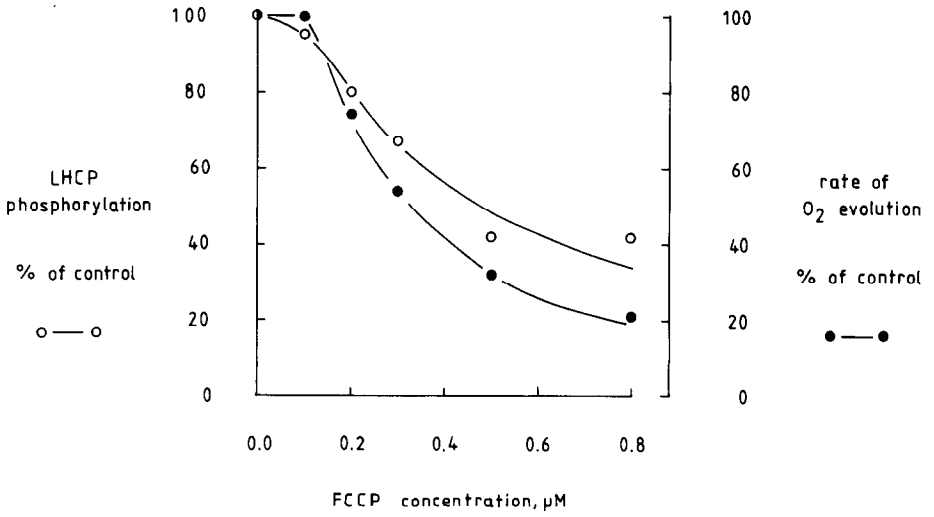


Fig.3. Titration with FCCP of the maximum level of ^{32}P incorporation into LHCP and of the maximum rate of accompanying CO_2-dependent O_2 evolution in intact pea chloroplasts. Control vlues are 475 cpm for LHCP phosphorylation and 41 μmol·mg $Chl^{-1}\cdot h^{-1}$ for O_2 evolution.

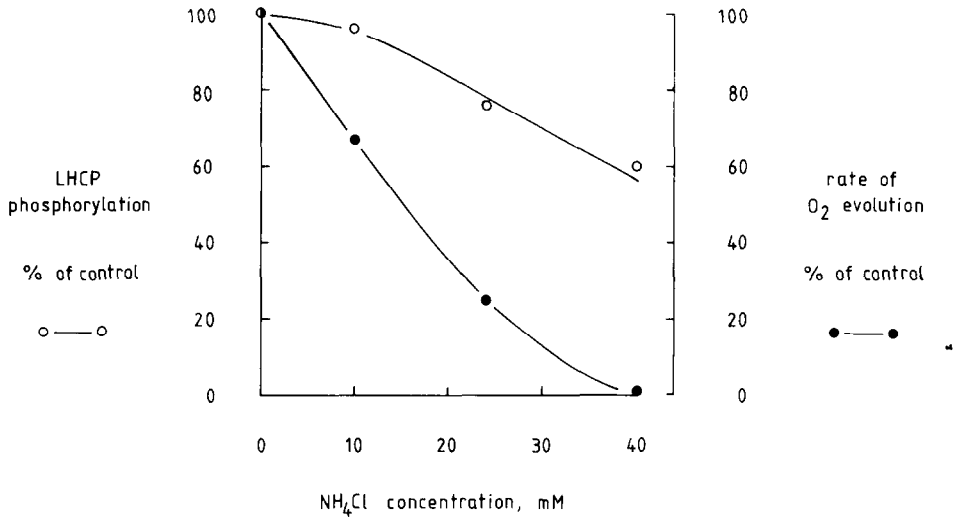


Fig.4. Titration with NH_4Cl of the maximum level of ^{32}P-incorporation into LHCP and of the maximum rate of accompanying CO_2-dependent O_2 evolution in intact pea chloroplasts. Control values are 290 cpm for LHCP phosphorylation and 36 μmol·mg $Chl^{-1}\cdot h^{-1}$ for O_2 evolution.

differs from fig.1A only in that the two sets of measurements were carried out on different chloroplast preparations. In both cases the maximum level of LHCP phosphorylation was observed after about 2 min illumination, and the level declined with the onset of CO_2-dependent O_2 evolution. Fig.2B shows the effect of 10 mM NH_4Cl in extending the lag-phase of CO_2-dependent O_2 evolution and in inhibiting the final rate by 33%. The initial rate of ^{32}P-incorporation was again decreased, though the maximum level of ^{32}P-incorporation was not greatly affected (<5% inhibition). Fig.2C shows the effects of NH_4Cl at 40 mM: CO_2-dependent O_2 evolution was essentially abolished, while the level of ^{32}P-incorporation into LHCP was inhibited only by about 40%. This single observation (fig.2C) may be taken as evidence that LHCP phosphorylation is possible even where CO_2-fixation is prevented by partial uncoupling of photophosphorylation.

Fig.3 shows a compilation of the FCCP-titration data (fig.1 together with 3 other sets of measurements). Fifty percent inhibition of total ^{32}P-incorporation into LHCP is seen by interpolation to occur at about 0.50 μM FCCP, while 50% inhibition of CO_2-dependent O_2 evolution occurs at about 0.34 μM FCCP. Fig.3 indicates that CO_2-dependent O_2 evolution is at least as sensitive as LHCP phosphorylation to inhibition by FCCP.

Fig.4 shows a compilation of the NH_4Cl-titration data (fig.2 with one other set of measurements). Extrapolation suggests that NH_4Cl at about 50 mM is required for 50% inhibition of ^{32}P-incorporation into LHCP, while 50% inhibition of CO_2-dependent O_2 evolution occurs at about 15 mM NH_4Cl. CO_2-dependent O_2 evolution is clearly more sensitive than LHCP phosphorylation to inhibition by NH_4Cl.

4. DISCUSSION

Published effects of FCCP and NH_4Cl on CO_2-dependent O_2 evolution in intact spinach chloroplasts [15] are in agreement with those recorded here and may also be explained as effects of uncoupling agents.

Control of protein kinase activity by the ATP/ADP ratio has been demonstrated in a reconstituted chloroplast system: acting as an ATP-sink, ribose-5-phosphate caused steady-state LHCP phosphorylation to decline [16]. LHCP phosphorylation could also be shown to be affected by plastoquinone redox state because of inhibition by the electron acceptor 3-phosphoglycerate [16].

For intact chloroplasts, the specific activity of the stromal ATP pool will depend on uptake of carrier-free $^{32}P_i$ from the medium (phosphate translocator activity) as well as on the rate of photophosphorylation. Both these factors would probably tend to accentuate uncoupler-inhibition of ^{32}P-labelling of LHCP, and it is possible that they contributed to uncoupler-inhibition of the initial rate of ^{32}P-incorporation in fig.1,2. Any consequent overestimation of the inhibitory effects of uncouplers on LHCP phosphorylation would tend to strengthen rather than to detract from the conclusion reached: CO_2-dependent O_2 evolution shows greater sensitivity to uncoupler-inhibition than does phosphorylation of LHCP.

A further objection can be levelled at the idea of excitation energy distribution being linked to the stromal ATP/ADP ratio: by positive feedback, any imbalance in the relative production of ATP and NADPH would be continually made worse if protein kinase activity were significantly dependent on the ATP supplied by photophosphorylation. If too much ATP were being produced, then the high ATP/ADP ratio would promote phosphorylation of LHCP and hence favour distribution of excitation energy to photosystem 1. Increased cyclic electron flow would then tend to increase ATP synthesis relative to reduction of $NADP^+$, leading to a greater imbalance and to further inhibition of the overall rate of photosynthesis. Conversely, if ATP synthesis were for any reason the chief limiting factor for photosynthesis then a decrease in protein kinase activity would exacerbate the problem by causing a diversion of excitation energy away from photosystem 1 and from cyclic photophosphorylation.

Physiological regulation of LHCP phosphorylation by plastoquinone redox state is more attractive in that it would tend to correct imbalances in the ratio of ATP to NADPH by a negative feedback control. Thus if ATP production exceeded its consumption, then the limiting factor for photosynthesis would become reduction of $NADP^+$. Plastoquinone would then tend to be oxidised since the excess of $NADP^+$ over NADPH would cause rapid turnover of photosystem 1. This oxidation of plastoquinone would switch off the protein kinase, and LHCP dephosphorylation would increase turnover of photosystem 2, thereby supplying reducing equivalents to make good the limiting rate of $NADP^+$ reduction. Conversely, too low an ATP/ADP ratio would be expected to inhibit NADPH oxidation by the CO_2-fixation pathway. Slow turnover of photosystem 1 would lead to reduction of plastoquinone, and hence to activation of the protein kinase and to phosphorylation of LHCP. Redistribution of excitation energy to photosystem 1 would then favour cyclic photophosphorylation, thereby tending to correct the initial deficiency of ATP.

Phosphorylation of LHCP and its control of excitation energy distribution may thus be involved under certain circumstances in adjustment of the ratio of cyclic to non-cyclic photophosphorylation. However, redox poising of the cyclic chain has been shown to be an important factor in regulation of cyclic photophosphorylation under physiological conditions [17,18] (review [4]). For maximum efficiency to be maintained, any change in the relative contribution of cyclic to total photophosphorylation will require a corresponding change in excitation energy distribution between the photosystems. Plastoquinone-controlled LHCP phosphorylation is perhaps the most likely means by which this could be achieved.

ACKNOWLEDGEMENTS

The experiments were carried out in the Plant Biochemistry Laboratory, Department of Biological Sciences, University of Warwick, where the author held a postdoctoral research fellowship funded by the SERC.

REFERENCES

[1] Allen, J.F., Bennett, J., Steinback, K.E. and Arntzen, C.J. (1981) Nature 291, 25–29.
[2] Horton, P. and Black, M.T. (1980) FEBS Lett. 119, 141–144.
[3] Allen, J.F. (1983) Trends Biochem. Sci. 8, 369–373.
[4] Allen, J.F. (1983) CRC Crit. Rev. Plant Sci. 1, 1–22.
[5] Barber, J. (1983) Photobiochem. Photobiophys. 5, 181–190.
[6] Bennett, J. (1983) Biochem. J. 212, 1–13.
[7] Horton, P. (1983) FEBS Lett. 152, 47–52.
[8] Haworth, P., Kyle, D.J., Horton, P. and Arntzen, C.J. (1982) Photochem. Photobiol. 36, 743–748.

[9] Horton, P., Allen, J.F., Black, M.T. and Bennett, J. (1981) FEBS Lett. 125, 193–196.
[10] Allen, J.F. and Horton, P. (1981) Biochim. Biophys. Acta 638, 290–295.
[11] Millner, P.A., Widger, W.E., Abbott, M.S., Cramer, W.A. and Dilley, R.A. (1982) J. Biol. Chem. 257, 1736–1742.
[12] Markwell, J.P., Baker, N.R. and Thornber, J.P. (1982) FEBS Lett. 142, 171–174.
[13] Baker, N.R., Markwell, J.P. and Thornber, J.P. (1982) Photobiochem. Photobiophys. 4, 211–217.
[14] Allen, J.F. and Bennett, J. (1981) FEBS Lett. 123, 67–70.
[15] Rosa, L. and Whatley, F.R. (1981) Plant Physiol. 68, 364–370.
[16] Horton, P. and Foyer, C. (1983) Biochem. J. 210, 517–521.
[17] Heber, U., Egneus, H., Hanck, U., Jensen, M. and Koster, S. (1978) Planta 143, 41–49.
[18] Ziem-Hanck, U. and Heber, U. (1980) Biochim. Biophys. Acta 591, 266–274.