Biochimica et Biophysica Acta 851 (1986) 166–172
Elsevier

BBA 41938

Thiol modulation of chloroplast CF_0-CF_1 in isolated barley protoplasts and its significance to regulation of carbon dioxide fixation

W. Paul Quick and John D. Mills

Department of Biological Sciences, University of Keele, Keele, Staffordshire, ST5 5BG (U.K.)

(Received February 24th, 1986)

Key words: Thiol modulation; ATPase; Photophosphorylation; CO_2 fixation; Thioredoxin; (Barley chloroplast)

(1) The extent of thiol-modulation of chloroplastic CF_0-CF_1 in isolated barley protoplasts was determined by their capacity to hydrolyse ATP in a subsequently lysed assay system and was measured under conditions that vary the rate of CO_2 fixation. CF_0-CF_1 activity could be separated from other ATPases by using the specific inhibitors triphenyltin and tentoxin. (2) Disruption of dark-adapted protoplasts resulted in little or no ATPase activity associated with CF_0-CF_1. Preillumination of the protoplasts induced CF_0-CF_1-dependent ATPase activity which was reversible in the dark. (3) Experiments using methyl viologen established that induction of CF_0-CF_1-dependent ATPase activity resulted from thiol-modulation of oxidised enzyme complexes by the endogenous thioredoxin system. (4) CF_0-CF_1 was shown to be rapidly thiol-modulated upon illumination ($t_1/_2 \approx 15$ s) well before steady-state rates of CO_2-dependent O_2 evolution were achieved. (5) The steady-state extent of light-induced thiol-modulation was shown to be light-saturated at 10 $W \cdot m^{-2}$, a light intensity far below that required to saturate the rate of CO_2-dependent O_2 evolution. (6) These results are consistent with the idea that thiol modulation of CF_0-CF_1 acts primarily as an on-off switch in vivo to prevent ATP hydrolysis in the dark.

Introduction

The membrane-bound protonmotive ATPase, CF_0-CF_1, of thylakoids isolated from dark-adapted leaves is not observed to catalyse net ATP hydrolysis upon addition of ATP. However, ATP hydrolysing activity is induced by preillumination in the presence of reduced thiols [1,2]. Since ATP synthesis is readily observed under the appropriate conditions and since the enzyme would be expected to catalyse this reaction reversibly, the lack of observed ATP. hydrolysis has been explained in terms of an overall deactivation of CF_0-CF_1 under conditions when ATP hydrolysis would be expected [2,6].

It is proposed that an electrochemical potential difference of protons ($\Delta\mu_{H^+}$) is required across the thylakoid membrane to produce a catalytically active CF_0-CF_1 [2,7]. Activation of CF_0-CF_1 occurs when $\Delta\mu_{H^+}$ is generated by electron transport [2,4,6] or by artificially imposed pH [3,5] and electrical gradients [5,7] across the thylakoid membrane. Preillumination of thylakoids in the presence of reduced thiols (typically dithiothreitol) is proposed to lower the magnitude of $\Delta\mu_{H^+}$ required to activate CF_0-CF_1 [3,5]. The reasons for this are not clear but the changes appear to be caused by the net reduction by dithiothreitol of a disulphide bridge on the γ subunit of CF_1 [19].

When CF_0-CF_1 is in the oxidised state the $\Delta\mu_{H^+}$

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; P_i, inorganic phosphate; PS I, Photosystem I.

required for activation exceeds that required for thermodynamic reasons to poise the catalytic reaction in the direction of net ATP synthesis. Hence, steady-state ATP hydrolysis is not possible when the enzyme is in the oxidised form, and can only proceed after thiol modulation has occurred when the magnitude of $\Delta\mu_{H^+}$ required for activation is lower than the $\Delta\mu_{H^+}$ required for ATP synthesis.

The rate of ATP synthesis catalysed by CF_0-CF_1 is thought to be a function of both the $\Delta\mu_{H^+}$ and the number of active CF_0-CF_1 enzymes [7]. As thiol modulation of CF_0-CF_1 lowers the $\Delta\mu_{H^+}$ for activation [3], then at a given $\Delta\mu_{H^+}$, there will be an increased number of active enzymes and hence increased rates of catalysis by thiol-modulated enzymes. It has been observed that thiol modulation of isolated thylakoids leads to enhanced rates (approx. 30%) of photophosphorylation [4,6], or a lowering of the $\Delta\mu_{H^+}$ (up to 0.3 pH units) required to achieve similar rates of acid/base induced ATP synthesis [3,5].

Thiol modulation of CF_0-CF_1 in vivo is thought to be achieved via the thioredoxin system [8,9]. The role of thiol modulation of CF_0-CF_1 has been proposed to act primarily as an on/off switch to prevent wasteful ATP hydrolysis occurring in the dark and to allow efficient ATP synthesis during illumination [3,4,8,9]. However, the extent of thiol modulation of CF_0-CF_1 could have a regulatory function during photosynthesis by varying the amount of thiol-reduced CF_0-CF_1 and hence the efficiency of photophosphorylation. To test these possibilities we examined the extent of thiol modulation of CF_0-CF_1 in Barley protoplasts. Protoplasts provide a compromise between the true in vivo state, such as intact leaves, which are difficult to manipulate experimentally, and isolated chloroplasts which are amenable to experimentation but which are not in their natural environment of the cell [10]. Preliminary experiments have shown that preillumination of isolated protoplasts induces net ATP hydrolysis by CF_0-CF_1 observed in a subsequently lysed assay system [11]. In this paper we have studied the kinetics of the thiol modulation process under conditions that vary the rate of CO_2 fixation. The results are consistent with the idea that thiol modulation of CF_0-CF_1 is primarily an on/off switch designed to prevent dark ATP hydrolysis.

Materials and Methods

Barley plants (*Hordeum vulgare*, var. Panda) were grown in vermiculite in a growth cabinet illuminated with fluorescence strip lights with a 16/8 h day/night cycle and a 25/20 °C day/night temperature cycle. The seedlings were harvested when they were approximately 15 cm high (about 7–10 days old). Protoplasts were prepared by enzymic digestion according to the method of Edwards et al. [10], except that the last 30 min of the 3 h digestion period was carried out in complete darkness. The protoplasts were purified by sucrose flotation and stored on ice. Protoplasts were dark adapted for at least 1 h prior to use. Intact chloroplasts were isolated from protoplasts according to the method of Leegood and Walker [12]. The percentage of intact chloroplasts as measured by the ferricyanide technique [13] was greater than 90% intact and the rate of CO_2-dependent O_2 evolution was typically the same as the parent protoplasts.

Intact protoplasts were preincubated in an oxygen electrode in a medium containing 0.5 M sorbitol, 5 mM $NaHCO_3$, 1 mM $MgCl_2$ and 30 mM Tris-KOH (pH 7.6). Lysed protoplasts were preincubated in a medium containing 0.33 M sorbitol, 20 mM KCl, 5 mM $MgCl_2$, 1100 units catalase per ml, 0.1 mM methyl viologen, 25 mM Tricine (pH 8.0) and 7.5 mM dithiothreitol as indicated. Lysis of the protoplasts was achieved by protoplast addition prior to the sorbitol and passing the suspension through a 10 μm mesh three times. Intact chloroplasts were preincubated in a medium containing 0.33 M sorbitol, 5 mM $NaHCO_3$, 2 mM EDTA, 1100 units catalase per ml and 10 mM Hepes buffer (pH 7.6). Lysed chloroplasts were obtained by adding the intact chloroplasts prior to sorbitol in the chloroplast preincubation medium; 0.1 mM methyl viologen and 7.5 mM dithiothreitol was also included.

Preincubation was carried out either in the dark or under blue illumination provided by a Corning 4.96 glass filter in an oxygen electrode so that the rate of CO_2-dependent O_2 evolution could be determined. 150 μl samples were removed at various times during the preincubation and assayed for ATP hydrolysis in a medium containing 2.5 mM ATP, 1.25 mM NH_4Cl, 20 mM Tris-KOH (pH

8.0) as described previously [9]. The rate of ATP hydrolysis was estimated from the amount of P_i released which was found to be reliable and quantitative when intact chloroplasts are used [9]. Tentoxin was obtained from the Sigma Chemical Company, Poole, Dorset, U.K.

Results

The potential hydrolysing activity of CF_0-CF_1 in intact protoplasts was assayed by a two stage procedure. Dark-adapted protoplasts were initially suspended at 100 μg chlorophyll per ml in an isotonic medium in an O_2 electrode and either dark-adapted or illuminated for 4 min. During this preincubation stage, small aliquots of protoplasts were sampled and added to an ATP containing medium for assay of ATP hydrolysis in the dark. Disruption of the protoplasts was induced by the hypotonicity of the assay medium and was accelerated by rapid passage of the protoplast/assay medium through a 10 μm nylon mesh as previously described [11]. Table I shows the amounts of P_i released by thylakoids from disrupted protoplasts after 4 min incubation in ATP hydrolysis medium. The first row represents controls where trichloroacetic acid, used to stop the reaction, was added prior to the protoplasts and shows that there was considerable P_i present that primarily resulted from the P_i content of the protoplasts. The remaining results show the amount of P_i formed after 4 min ATP hydrolysis in the assay stage. After 4 min the amount of P_i was increased for both dark- and light-preincubated samples, but was higher in the latter. This increase is due to ATP hydrolysis in the assay stage. The apparent difference between the amount of ATP hydrolysed by light- and dark-preincubated samples was inhibited by both triphenyltin, an F_0-F_1 type ATPase inhibitor, and tentoxin, a specific inhibitor of CF_0-CF_1. The activity not inhibited by triphenyltin or tentoxin is probably associated with non F_0-F_1 type ATPases and phosphatases. This activity remains constant for both light and dark-adapted protoplasts [11] demonstrating that the light-induced increase in P_i is associated with ATP hydrolysis by CF_0-CF_1. Although background levels of phosphate varied between preparations the light induced changes in activity were reproducible to within 5% [11].

Fig. 1 shows the rate of ATP hydrolysis by samples of disrupted protoplasts taken at various times after illumination and during a subsequent dark period. Illumination caused an increase in the rate of ATP hydrolysis that was largely complete after 30 s ($t_{1/2} \approx 15$ s). This rate remained

TABLE I

μmol P_i RELEASED PER MG CHLOROPHYLL BY THYLAKOIDS FROM DISRUPTED PROTOPLASTS AFTER 4 MIN INCUBATION IN ATP-HYDROLYSIS MEDIUM

0.5 ml of 5% trichloroacetic acid was used to terminate the reaction; the control had trichloroacetic acid added prior to addition of the thylakoids. Tentoxin and triphenyltin (TPT) were added to a final concentration of 10 μg/ml and 10 μM, respectively.

	Dark	Light
Control	10.1	9.9
4 min incubation	21.1	26.4
4 min incubation + tentoxin	19.5	18.7
4 min incubation + TPT	18.5	18.8

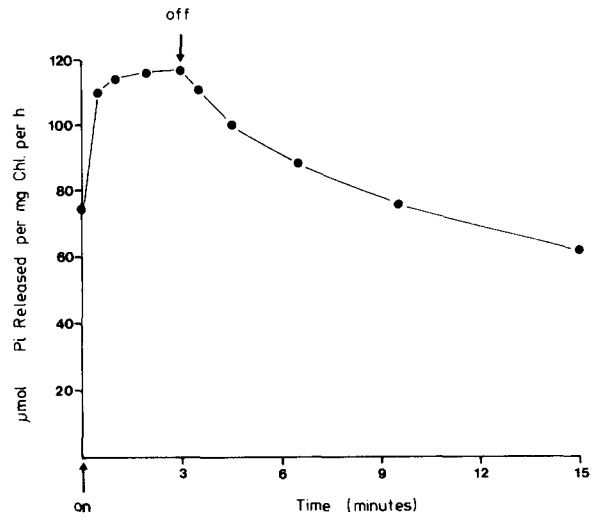

Fig. 1. Effect of preillumination on the rate of P_i released by samples of Barley protoplasts subsequently lysed by addition to an ATP-containing medium. The rates of P_i released are expressed minus the TCA control to remove the high background P_i. The arrows indicate the time period during which the princubation medium was illuminated.

constant until the light was turned off. The subsequent dark period caused a decline in the rate of ATP hydrolysis with a $t_{1/2}$ of approx. 3 min. This demonstrates the reversible nature of the light activation of ATP hydrolysis by CF_0-CF_1 and is essentially identical to observations made with isolated, non-CO_2 fixing, intact Pea chloroplasts [16].

Fig. 2 shows the rate of CF_0-CF_1 induced ATP hydrolysis measured from samples taken after various times of illumination. CF_0-CF_1-dependent activity was estimated by the total activity minus the tentoxin control. Fig. 2 also shows the CO_2-dependent rate of O_2 evolution (in brackets) at different intensities of illumination. Varying light

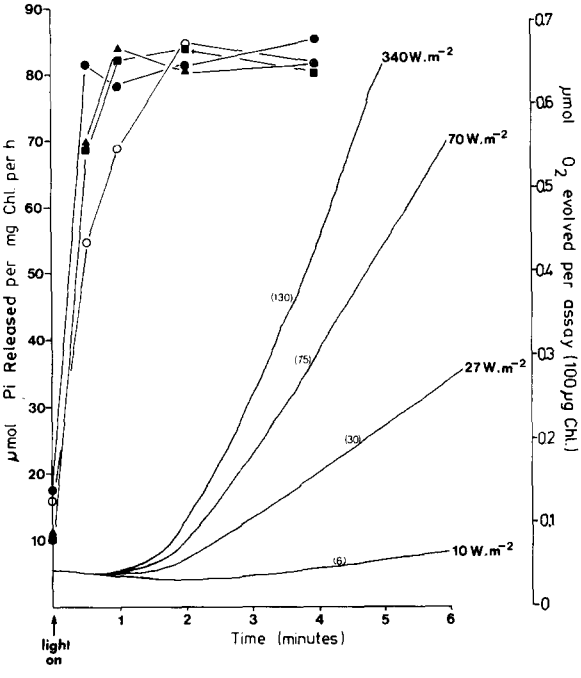

Fig. 2. Comparison of the induction kinetics of CO_2 fixation and ATP hydrolysis activity in intact protoplasts. The rates (in brackets) of CO_2-dependent O_2 evolution (solid lines, no symbols) are shown as measured during the preincubation period for Barley protoplasts at four different light intensities. Also shown is the rate of P_i released by samples of protoplasts taken at various times during the preincubation period at the different light intensities (-340 W·m^{-2}; -70 W·m^{-2}; -27 W·m^{-2}; -10 W·m^{-2}). The rate of P_i released was determined (after lysis) as the observed level of P_i released minus a control containing Tentoxin to show only the CF_0-CF_1 induced activity. The rate of CO_2 dependent O_2 evolution is expressed as μmol O_2 evolved per mg chlorophyll per h.

intensity had dramatic effects on the rate of O_2 evolution but very little effect on the kinetics or magnitude of the rate of ATP hydrolysis. For all light intensities used (10–340 W·m^{-2}) the increase in rate of ATP hydrolysis due to illumination was constant and was largely complete after 1 min of light incubation. The rate of CO_2-dependent O_2 evolution showed a characteristic lag phase taking approx. 3 min before steady-state rates were achieved. The rate of ATP hydrolysis and hence the extent of thiol modulation of CF_0-CF_1 seems to be independent of the final rate of CO_2 evolution and seems to be largely complete before the onset of CO_2-dependent O_2 evolution. In Fig. 3 the extent of the light-induced increase in the rate of ATP hydrolysis and the steady-state rate of CO_2-dependent O_2 evolution is shown for an extended range of light intensities. At 3 W·m^{-2} the steady-state rate of ATP hydrolysis is very low (approx. 10 μmol P_i per mg Chl per h) but at 10 W·m^{-2} nearly full activation is achieved. Between 10 and 1000 W·m^{-2} there is very little change in the steady-state rate of ATP hydrolysis. Thus, within the resolution of the experiment, thiol modulation of CF_0-CF_1 is virtually a step function of the light intensity between 3 and 10 W·m^{-2}. In contrast, the rate of CO_2 fixation is very low below 10 W·m^{-2} and increases more gradually over the entire light intensity range.

CF_0-CF_1 is activated by $\Delta\mu_{H^+}$ and thiol modulation of CF_0-CF_1 is observed to lower the $\Delta\mu_{H^+}$ required for activation allowing the enzyme to hydrolyse ATP [3,7]. The light-dependent increase in ATP hydrolysing activity discussed so far does not distinguish between $\Delta\mu_{H^+}$ activation of prereduced enzyme and light induced thiol reduction of oxidized CF_0-CF_1. In isolated intact chloroplasts, it has been observed that the oxidation of CF_0-CF_1 occurs within 5 min of dark adaption [16]. This was shown by reilluminating chloroplasts in the presence of the PS I acceptor, methyl viologen, which enhances the generation of a $\Delta\mu_{H^+}$ but prevents reduction of thioredoxin by the electron transport chain [16]. Thus, reillumination in the presence of methyl viologen will activate prereduced CF_0-CF_1, giving ATP hydrolysis but will not allow thiol modulation of oxidized enzyme complexes (no ATP hydrolysis).

To investigate whether dark adapted proto-

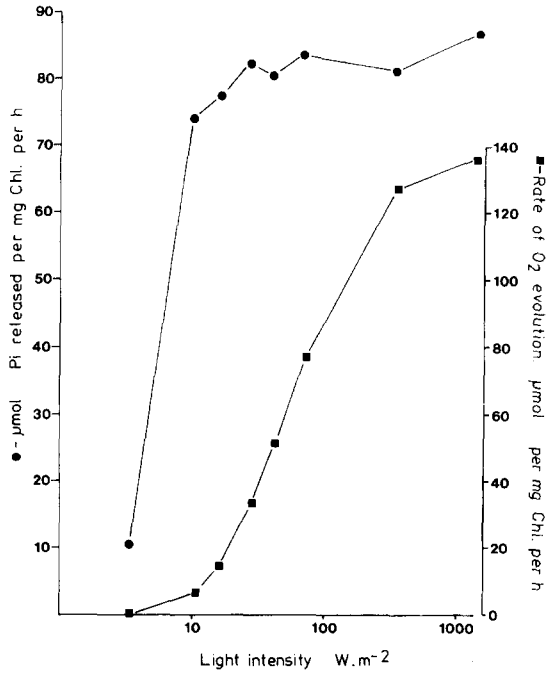

Fig. 3. The rate of P_i released by lysed samples of barley protoplasts taken after 4 min preincubation is shown for several intensities of illumination during the preincubation. Also shown is the steady-state rate of CO_2-dependent O_2 evolution achieved during the preincubation period. The rate of P_i released is expressed as the observed rate minus a tentoxin containing control and hence reflects only CF_0-CF_1 activity.

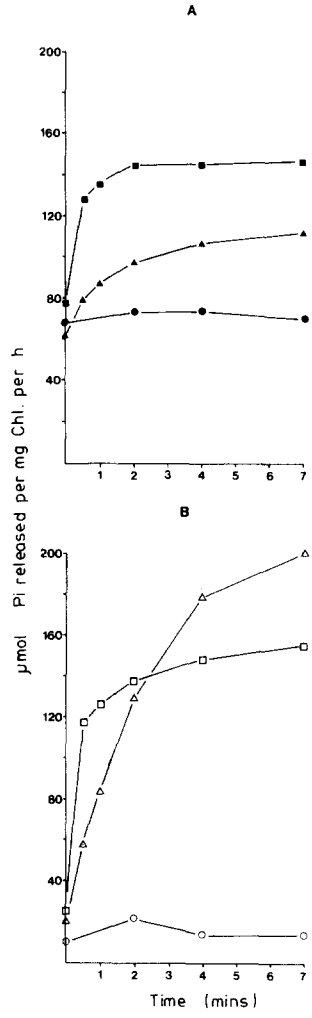

Fig. 4. The rate of P_i released by lysed samples of protoplasts (A) and chloroplasts isolated from protoplasts (B) taken after various times of preincubation. The square symbols represent protoplasts or chloroplasts preincubated intact and displaying CO_2-dependent O_2 evolution. Triangles represent protoplasts or chloroplasts that were lysed during the preincubation period and had 7.5 mM dithiothreitol and 100 μM methyl viologen included into the preincubation medium. Circles represent conditions similar to the triangle except the addition of dithiothreitol was omitted.

plasts contain CF_0-CF_1 in a pre-reduced state, experiments were carried out using methyl viologen. It was observed that 100 μM methyl viologen did not inhibit the light-dependent increase in ATP hydrolysing activity (results not shown). However, this concentration of electron acceptor had not effect on the rate of CO_2 fixation suggesting that protoplasts are relatively impermeable to methyl viologen. The effect of methyl viologen was therefore investigated in freshly lysed protoplasts.

Fig. 4a shows the typical light-induced increase in ATP hydrolysing activity observed with intact protoplasts in the absence of methyl viologen. Lysed protoplasts showed no such light-induced increase in the presence of methyl viologen alone. This indicates that CF_0-CF_1 was largely in the oxidized state. After the addition of dithiothreitol to the lysed system, a light-induced increase in ATP hydrolysing activity was observed. Thus it seems reasonable to conclude that CF_0-CF_1 is largely oxidized in dark-adapted protoplasts.

Fig. 4b shows a similar experiment to Fig. 4a, except that intact chloroplasts rapidly isolated from protoplasts were used. Intact (more than 90%) and lysed chloroplasts showed similar kinet-

ics to intact and lysed protoplasts. However, the extent of light-induced activation was increased from 65 to 140 in intact and 50 to 180 in lysed protoplasts and chloroplasts respectively. This suggests that CF_0-CF_1 activity is suppressed by more than 50% in protoplasts compared to chloroplasts isolated from protoplasts. This was found to be associated with an inhibitory substance released during rupture of the protoplasts. The method of isolating chloroplasts involves centrifugation and a separation of a chloroplast pellet from the rest of the aqueous cell contents. This implies that the inhibitor is solubilized upon rupture of the protoplasts. This was demonstrated by experiments where protoplasts and chloroplasts were mixed. Table II shows the rate of ATP hydrolysis for samples of intact chloroplasts that were either dark or light adapted. The light-induced increase in the rate of ATP hydrolysis in protoplasts was approx. 50% of the chloroplast value. Incubation of protoplasts and chloroplasts together showed a light-induced increase in the rate of ATP hydrolysis but this was less than the sum of the rates achieved by chloroplasts and protoplasts individually, implying that the lysed protoplasts were inhibiting chloroplastic CF_0-CF_1 activity.

TABLE II

THE RATE OF P_i RELEASED (μmol P_i RELEASED PER mg Chl PER h) FROM ATP BY SAMPLES OF THYLAKOIDS TAKEN FROM PROTOPLASTS OR INTACT CHLOROPLASTS OR A MIXTURE OF BOTH, THAT WERE PREINCUBATED FOR 4 MIN IN THE PRESENCE OF ABSENCE OF 400 $W \cdot m^{-2}$ BLUE ILLUMINATION (GIVEN BY A CORNING 4-96 GLASS FILTER)

Protoplasts and chloroplasts were preincubated at 100 μg chlorophyll perml. The mixture of protoplasts and chloroplasts (row 3) contained 200 μg of chlorophyll per ml; 100 μg of each. Row 4 shows the expected result of row 3 by the addition of rows 1 and 2.

	Dark	Light	Light-induced increase
1. Intact protoplasts	67	134	67
2. Intact chloroplasts	26	165	139
3. Intact protoplasts + intact chloroplasts	116	232	116
4. The sum of row 1 and row 2	93	299	206

Addition of a soluble protoplast extract (obtained by mechanical rupturing and centrifugation) to the ATP hydrolysis medium caused marked inhibition of the rate of ATP hydrolysis. Dialysis of the extract relieved the inhibition, but inhibition was uneffected by trypsin digestion of the extract. The inhibitor is presumed to be a small soluble compound and could be phenolic compounds present in the cell vacuole that would be released upon rupture of the protoplasts. The presence of this inhibitor can account for the reduced rates of ATP hydrolysis by thylakoids from protoplasts compared to the intact chloroplasts.

Isolation of the cloroplasts from the protoplasts removed to a large extent the background levels of phosphate observed in ruptured protoplasts and also the non F_0-F_1 type ATPase or phosphatase activity. This observation supports the earlier assumption that these two components are probably located outside the chloroplastic compartment of the protoplast.

Discussion

This paper demonstrates that the well known thiol-modulation of CF_0-CF_1 observed in isolated intact chloroplasts [8,9,17] and chloroplasts rapidly isolated from intact leaves [18,19] is also observed in protoplasts. This allows the significance of the phenomenon to be investigated under conditions that closely resemble the in vivo state.

The kinetics of thiol modulation were similar to those observed in isolated intact chloroplasts and the modulation was reversible upon terminating illumination. The analysis of results are somewhat complicated in the protoplast system by the high P_i background and by the presence of non-F_0-F_1 type ATPases and other phosphatases. However, the amount of background P_i was shown to be independent of illumination and CF_0-CF_1-dependent ATP hydrolysis could be separated from other ATPases and phosphatases by the use of the specific inhibitors, triphenyltin and tentoxin. The major conclusion of this work is that both activation and thiol modulation of CF_0-CF_1 are complete well before the onset of maximal rates of CO_2 fixation over a variety of light intensities that vary the rate of CO_2 fixation by an order of magnitude. This indicates that thiol modulation of

CF_0-CF_1 does not regulate the rate of CO_2 fixation during the induction phase of CO_2 fixation.

The steady-state extent of activation of CF_0-CF_1 is maximal at light intensities far below those required to saturate CO_2 fixation. This indicates that thiol modulation of CF-CF_1 does not contribute to the regulation of the steady-state rate of CO_2 fixation under these conditions. This conclusion broadly agrees with studies of other thioredoxin-activated stromal enzymes [20–22].

The results support the earlier suggestion that thiol modulation of CF_0-CF_1 acts primarily as an on/off switch to prevent ATP hydrolysis in the dark [3]. This conclusion is strengthened by the observed steep dependence of the process on light intensity, with the extent of modulation varying from nearly zero to 100% over the range of 3–10 $W \cdot m^{-2}$, an intensity range which is associated with very low rates of CO_2 fixation. This is consistent with the idea that the redox state of CF_0-CF_1 is in equilibrium with the redox state of the thioredoxin pool of the chloroplast stroma. Thioredoxin can presumably be maintained in a reduced form by very low rates of electron transport due to the limited pool size and the slow rate of oxidation. This is in contrast to the electron acceptor NADP which is relatively rapidly oxidized by the operation of the Calvin-cycle.

It appears that our data strongly favours the on/off switch proposal as the function of the thiol modulation process. However, one observation may cast some doubt that the process does in fact have a role in regulating CO_2 fixation under different conditions. This observation is that the extent of thiol modulation in both intact protoplasts and intact chloroplasts isolated from protoplasts was less than the extent observed in lysed chloroplasts reduced by dithiothreitol. This observation cannot be wholly explained by the presence of inhibitor demonstrated here to be relased upon disruption of protoplasts. It also cannot be explained by dithiothreitol-induced reduction of the broken chloroplasts fraction in the intact chloroplast preparation, since the chloroplasts were more than 90% intact. We currently have no explanation for this discrepancy but it may suggest that the maximal extent of thiol modulation in the intact system is less than 100%. Further experiments are in progress to investigate this phenomenon.

Acknowledgements

We thank S. Hill and J.R. Best for their capable technical assistance, and M.A. Cowen and G.B. Burgess for help in the preparation of the manuscript. We are grateful to the SERC for financial support of this work.

References

1 Petrack, B., Craston, A., Sheppy, F. and Farron, F. (1965) J. Biol. Chem. 240, 906–914
2 Bakker-Grunwald, T. and Van Dam, K. (1974) Biochim. Biophys. Acta 347, 290–298
3 Mills, J.D. and Mitchell, P. (1982) FEBS Lett. 144, 63–67
4 mills, J.D. and Mitchell, P. (1984) Biochim. Biophys. Acta 764, 93–104
5 Junesch, U. and Graber, P. (1985) Biochim. Biophys. Acta 809, 429–434
6 Rumberg, B. and Becher, U. (1984) in H^+-ATPase: Structure, Function, Biogenesis (Papa, S. et al., eds.), pp. 421–430, Adriatica Editrice, Bari
7 Schlodder, E., Graber, P. and Witt, H.T. (1982) in Electron Transport and Photophosphorylation (Barber, J., ed.), Topics in Photosynthesis, Vol. 4, pp. 105–175, Elsevier Biomedical Press, Amsterdam
8 Mills, J.D., Mitchell, P. AND SCHURMANN in PRoceedings of the Vth International Congress on Photosynthesis at halkidiki (Akoyonoglou, G., ed.), pp. 839–848, Balaban Science Services, Philadelphia, PA
9 Milles, J.D., Mitchell, P. and Schurmann, P. (1980) FEBS Lett. 112, 173–177
10 Edwards, G.E., Robinson, S.P., Tyler, N.J.C. and Walker, D.A. (1978) Plant Physiol. 62, 313–319
11 Mills, J.D. and Quick, W.P. (1986) Biochem. Soc. Trans. 14, 20–22
12 Leegood, R.C. and Walker, D.A. (1981) Plant Cell and Environment 4, 59–66
13 Lilley, R.McC., Chon, C.J., Mosbach, A. and Heldt, H.W. (1977) Biochim. Biophys. Acta 460, 259–272
14 Gould, J.M. (1976) Eur. J. Biochem. 62, 567–575
15 Steele, J.A., Uchytil, T.F. and Durbin, R.D. (1978) Biochim. BIophys. Acta 504, 136–141
16 Mills, J.D. and Mitchell, P. (1982) Biochim. BIophys. Acta 679, 75–83
17 Shahak, Y. (1982) Plant Physiol. 70, 87–91
18 marchant, R.H. (1980) in Abstracts of the Proceedings of Vth International Congress on Photosynthesis at Halkidiki (Akoyonoglous, G., ed.), pp. 365
19 Vallejos, R.H., Arana, J.L. and Ravizzini, R.A. (1983) J. Biol. Chem. 253, 7317–7321
20 Heber, U., Takahama, U., Neimanis, S. and Shimizu-Takahama, M. (1982) Biochim. Biophys. Acta 679, 287–299
21 Gardemann, A., Stitt, M. and Heldt, H.W. (1983) Biochim. BIophys. Acta 722, 51–60
22 Leegood, R.C. and Walker, D.A. (1980) Arch Biochem. Biophys. 200, 575–582