MODULATION OF FRUCTOSE BISPHOSPHATASE ACTIVITY IN INTACT CHLOROPLASTS

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1. Introduction

There is considerable evidence that several of the enzymes of the reductive pentose phosphate (RPP) pathway are affected by light. Enzyme activity which is promoted by a relatively alkaline pH and an increase in $[Mg^{2+}]$ is thus increased in the light and diminished in the dark [1–3]. In addition, certain enzymes undergo a process of reductive activation in the light which is dependent upon reductants generated by photosystem I [4]. Reductive activation of fructose 1,6-bisphosphatase (EC 3.1.3.11) (FBPase) is particularly striking [5]. Thus if chloroplasts or protoplasts are taken from the dark and lysed into an assay mixture containing ample Mg^{2+} at an alkaline pH, activity is extremely low, whereas illumination brings about a rapid increase in activity [5].

This paper reports results which indicate that the activity of FBPase in intact chloroplasts may be directly related to the availability to the activation system of photosynthetically generated electrons. When this availability is diminished, either by decreasing the rate of electron transport, by providing alternative electron sinks, or both, FBPase activity is also diminished.

2. Materials and methods

Protoplasts of wheat (*Triticum aestivum*) were prepared and purified as i [6]. Protoplasts were stored on ice for 3–4 h pr or to use. Chloroplasts were prepared by sedimenting protoplasts at 100 × *g* for 2 min and resuspending them in 400 mM sorbitol, 10 mM EDTA, 25 mM Tricine (pH 8.4). The suspension was passed 3 times through a 20 μm nylon mesh attached to a 1 ml plastic syringe and the chloroplasts separated by centrifugation at 250 × *g* for 45 s. Intactness of the chloroplasts was >90% when assessed by the ferricyanide method [7]. Chlorophyll was determined by the method in [8]. For measurement of enzyme activity, chloroplasts were incubated at 20°C in a medium containing 400 mM sorbitol, 10 mM EDTA, 25 mM Tricine (pH 8.4) and additions as indicated. Chlorophyll (chl) was 50–100 μg/ml and chloroplasts were illuminated [9] with red light (ICI Perspex Red 400) at an intensity of 330 W/m².

FBPase activity was measured at 20°C by mixing 50 μl samples of chloroplast suspension (containing 2.5–5 μg chl) with 0.95 ml of a reaction mixture containing 1 mM FBP, 1 mM EDTA, 0.4 mM $NADP^+$, 10 mM $MgCl_2$, 0.025% (v/v) Triton X-100, 4 units glucosephosphate isomerase and 2 units glucose 6-phosphate dehydrogenase in 100 mM Tris–HCl (pH 8.2) [10].

Antimycin A was obtained from Boehringer, Mannheim.

3. Results

When intact chloroplasts were illuminated, there was a marked activation of FBPase which reached its maximum activity after 5–10 min (fig.1, lower curve), an increase of some 4-fold. Fig.1 also shows that when the light was switched off, an inactivation occurred which was much more rapid than the activation process. In this experiment bicarbonate was present in the incubation medium. In experiments with bicarbonate-free chloroplasts, a considerably higher enzyme activity was realized when chloroplasts were illuminated. Fig.1 further illustrates the differences observed when FBPase activation was measured in the presence and absence of bicarbonate. While differences in the rate of activation were not marked, they were very apparent in the rate and extent of

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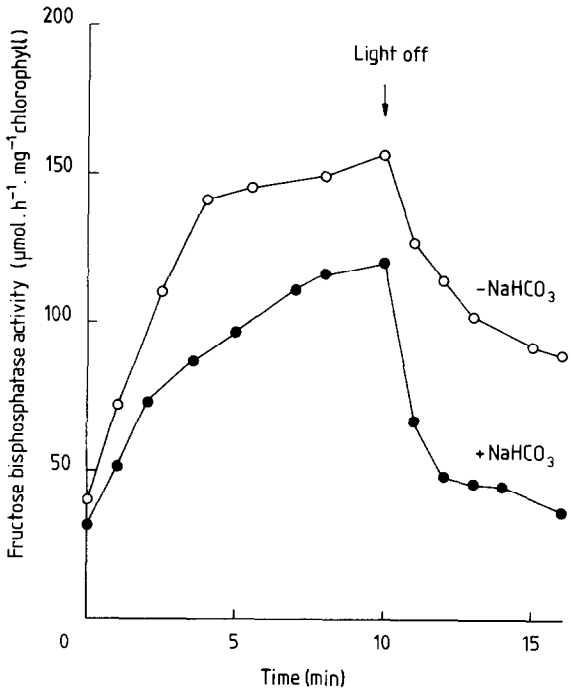

Fig.1. Light activation and dark inactivation of FBPase in the presence (•) and absence (○) of 10 mM $NaHCO_3$. Incubations also contained 0.2 mM P_i.

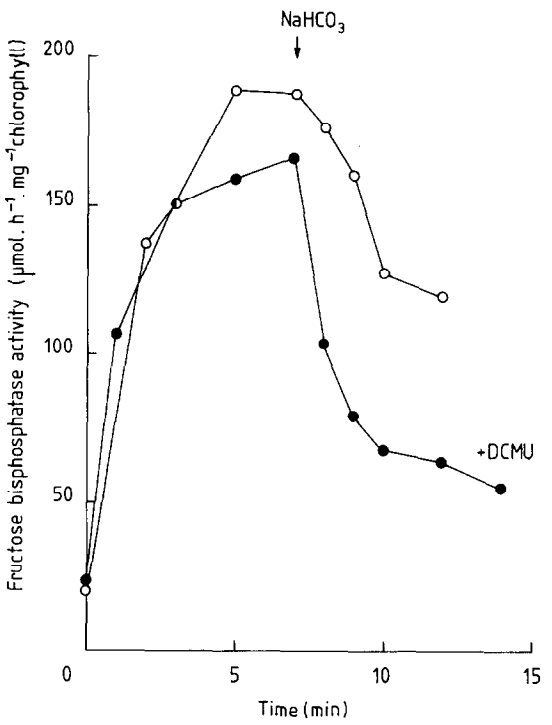

Fig.2. Effect of the addition of 10 mM bicarbonate on FBPase activity in chloroplasts illuminated in the presence (•) and absence (○) of 10^{-7} M DCMU. Rates of photosynthesis following the addition of $NaHCO_3$ were 47 and 100 μmol $O_2 . h^{-1} . mg\ chl^{-1}$ for chloroplasts with and without DCMU, respectively.

dark inactivation when bicarbonate was omitted. Thus in those chloroplasts incubated without bicarbonate, and with oxygen as the only apparent electron acceptor, FBPase activity had only returned to about half its maximum value after 6 min darkness, whereas in chloroplasts incubated with bicarbonate, FBPase activity had virtually returned to the level obtained at the start of illumination after only 2 min darkness.

The reduction in the activity of FBPase brought about by bicarbonate was also observed when it was added to bicarbonate-free chloroplasts in which the enzyme had already been light-activated (fig.2, upper curve). There was a rapid decrease in the activity of the enzyme from values (>150 $\mu mol . h^{-1} . mg\ chl^{-1}$) which were not normally observed when chloroplasts were reducing bicarbonate to more typical values (100–150 $\mu mol . h^{-1} . mg\ chl^{-1}$). These results suggest that bicarbonate may exert its effect on FBPase by virtue of being a good electron acceptor and that electrons which would be available for FBPase reduction in the absence of bicarbonate are then diverted to the reduction of bicarbonate. The effect of including 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), at a concentration which only inhibited photosynthesis by 50%, was also investigated. With DCMU present the effect of bicarbonate was much more striking (fig.1, lower curve) and FBPase activity declined rapidly to about the dark value.

If FBPase activity responds to the 'availability' of electrons, as the above results suggest, other electron acceptors should promote similar effects. When oxaloacetate, which is reduced in chloroplasts by an $NADP^+$-dependent malate dehydrogenase, was added to illuminated bicarbonate-free chloroplasts, there was a decline in FBPase activity resembling that caused by bicarbonate (fig.3). Since oxygen is also an electron acceptor, although a poor one [11], its concentration in illuminated, bicarbonate-free chloroplasts was also varied. No changes in FBPase activity were detected over 30–900 μM O_2.

Antimycin A was used to explore the converse of the above, i.e., that once activated in the presence

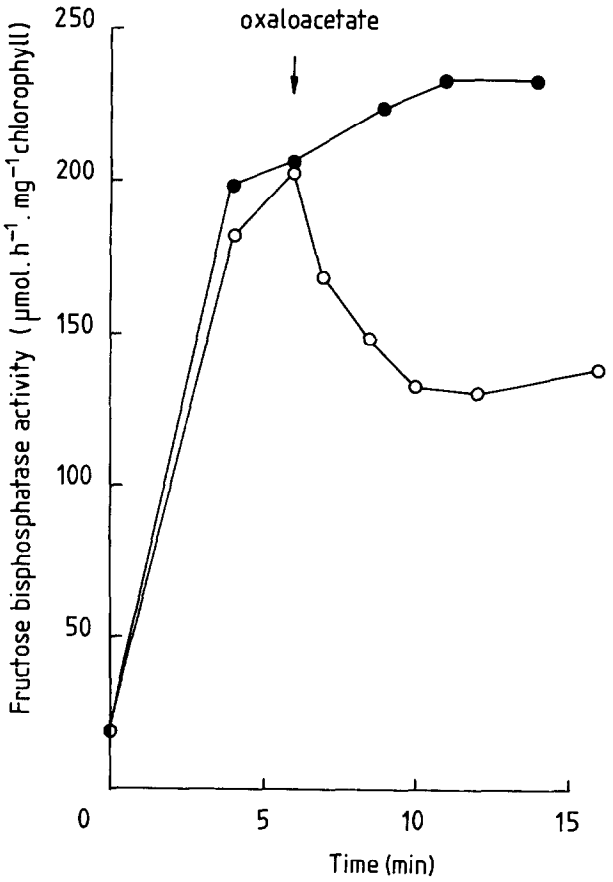

Fig.3. Effect of the addition of 2 mM oxaloacetate (○) on FBPase activity in chloroplasts illuminated in the absence of bicarbonate.

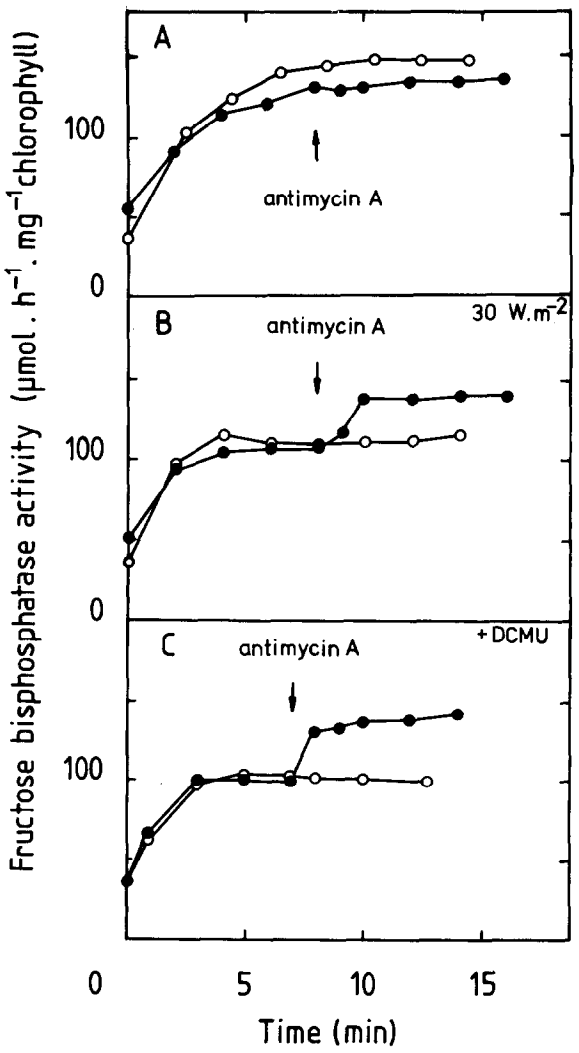

Fig.4. Influence of antimycin A on FBPase activity in illuminated chloroplasts (A), at low light intensity (B) and in the presence of 10^{-7} M DCMU (C). Reaction mixtures contained 10 mM $NaHCO_3$ and 0.2 mM P_i. Antimycin A was added in 1 µl ethanol to 1 µM final conc. (●). Ethanol (1 µl) was added to the controls (○). Light intensity was reduced by means of a neutral gauze filter to 30 W/m².

of an acceptor, FBPase activity might be increased if non-cyclic electron flow were increased. Antimycin A appears to be a potent inhibitor of cyclic electron transport in intact chloroplasts [12] and has effects which include measured increases in the activity of FBPase [13,14] or increases which are apparent from changes in substrates [15]. When optimal conditions were employed for chloroplasts illuminated in the presence of bicarbonate, antimycin A caused either no change or a slight increase in activity (fig.4), depending upon the chloroplast preparation. Only when conditions were made suboptimal, e.g., when electron flow was reduced by the addition of DCMU, or by a reduction in light intensity (fig.4), did larger increases in enzyme activity become apparent. In both cases, antimycin A restored activity to that observed in the uninhibited control.

4. Discussion

There is good evidence that reductive activation of FBPase is mediated by thioredoxin and its reductase, which is in turn reduced by electrons from ferredoxin [16]. It has been proposed that dark oxidation of the enzyme is brought about by oxidised glutathione [17]. While glutathione does cause changes in the

activation state of the purified enzyme [16], the available evidence suggests that in chloroplasts glutathione is present largely in the reduced form both in the light and the dark [18] and so would be unlikely to be effective in the process of dark inactivation.

These results also suggest that changes in the reduction status of ferredoxin might determine FBPase activity (cf. [13–15,19]) and that this carrier might also be directly involved in the dark inactivation process. If this supposition is correct, it would follow that any oxidant which could compete with the enzyme for ferredoxin-donated electrons would decrease its catalytic activity. Thus in the absence of an added electron acceptor, total reduction of FBPase probably occurs (the high activities measured in chloroplasts in the absence of an added electron acceptor are comparable with activities measured in crude extracts in the presence of dithiothreitol, which mimics the process of reductive activation). Addition of CO_2 or oxaloacetate did diminish FBPase activity (fig.1–3), particularly when electron flow was already diminished by the addition of DCMU (fig.2). Conversely, antimycin A stimulated FBPase under such conditions, in accord with its known ability to inhibit cyclic electron flow (which would tend to drain electrons from ferredoxin) and its tendency to stimulate CO_2 assimilation under certain limiting conditions [11,20].

The post-illumination stimulation of FBPase inactivation by CO_2 over that caused by oxygen (fig.1) presumably reflects the added drain on residual light-generated 'reducing power' occasioned by this substrate. Although other effects, such as changes in the stromal pH, might accompany the addition of substrates to illuminated chloroplasts, particularly those inhibited by DCMU [11], the evidence suggests that, once activated, the reduction status of the enzyme is not directly affected by changes in stromal pH or Mg^{2+} (R.C.L., D.A.W., unpublished). Whether the precise mechanism by which inactivation occurs is via a direct reversal of the thioredoxin activation system is currently being investigated.

Acknowledgement

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