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CO-OPERATIVE ACTIVATION OF CHLOROPLAST FRUCTOSE-1,6-BISPHOSPHATASE BY REDUCTANT, pH AND SUBSTRATE

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Intact chloroplasts capable of high rates of photosynthesis fail to reduce CO_2 when illuminated in the absence of oxygen. While anaerobiosis limits proton gradient formation leading to ATP deficiency (Ziem-Hanck, U. and Heber, U. (1980) Biochim. Biophys. Acta 591, 266–274), light activation of fructose-1,6-bisphosphatase was also inhibited by anaerobiosis, whereas light activation of NADP-malate dehydrogenase was stimulated by anaerobiosis, indicating that reductant was still available for light activation. The chloroplast pool of NADP was largely reduced during illumination under anaerobiosis and electron transport to oxaloacetate was not inhibited by anaerobic conditions. Significant light activation of fructose-bisphosphatase was observed in anaerobic chloroplasts with 3-phosphoglycerate as substrate, but not with dihydroxyacetone phosphate (3-phosphoglycerate supports electron transport and hence proton gradient formation). In the absence of added substrates, illumination of anaerobic chloroplasts resulted in some light activation of fructose-bisphosphatase when the pH of the medium was increased. Under these conditions, light activation was stimulated by dihydroxyacetone phosphate. Dihydroxyacetone phosphate added together with oxaloacetate allowed light activation of fructose-bisphosphatase in anaerobic chloroplasts, while neither substrate added alone was effective. Formation of a transthylakoid proton gradient can therefore substitute for an alkaline suspension medium by causing an alkaline shift of the stromal pH on illumination. The data are interpreted as indicating that fructose-bisphosphatase, but not NADP-malate dehydrogenase, requires an alkaline pH and the presence of substrate for rapid reductive light activation and they bear on the interpretation of the lag observed in photosynthesis in chloroplasts and leaves on illumination after a prolonged dark period.

Introduction

On illumination of darkened leaves or of chloroplasts isolated from darkened leaves, photosynthetic carbon fixation and its associated oxygen evolution is characterised by a lag phase of variable length [1]. Photosynthesis starts slowly and accelerates until a steady rate is achieved. Osterhout and Haas [2] attributed the lag to two possible factors, one the light activation of catalysts, the other an increase in the concentrations of key intermediates. While the available evidence suggests that pronounced lags (of several minutes duration) are not limited by the activation state of enzymes alone [3,4], lags in chloroplasts of the order of a minute or less are rather more difficult to interpret, since reductive activation of enzymes and increases in substrate concentrations, pH and

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Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

Mg^{2+} concentration occur simultaneously. Controversy also surrounds the degree to which factors influencing the lag phase in chloroplasts, for example, added substrates (such as 3-phosphoglycerate) or a high external orthophosphate concentration (which would deplete endogenous substrates via exchange through the phosphate translocator), influence reductive activation of enzymes such as fructose-bisphosphatase [3–6].

It has been known for many years that chloroplasts kept under strictly anaerobic conditions are unable to reduce CO_2 (see, e.g., Ref. 7). This has been explained by over-reduction of the electron-transport chain. Hence, the proton gradient formed by chloroplasts illuminated under anaerobic conditions is drastically reduced and chloroplast ATP/ADP ratios remain low in the light. In consequence the conclusion has been drawn that lack of ATP limits photosynthesis under anaerobic conditions [7]. Subsequently it has been shown that light activation of fructose-bisphosphatase proceeeds more slowly under anaerobiosis than at air levels of oxygen [8]. These observations are extended here. It will be shown that, in contrast to fructose-bisphosphatase, NADP-malate dehydrogenase is readily activated in chloroplasts illuminated in the absence of oxygen and that the inhibition of fructose-bisphosphatase activation can be attributed both to decreased alkalinisation of the stroma and to substrate deficiency. Evidence will also be presented that photosynthesis is inhibited under anaerobiosis even when fructose-bisphosphatase has previously been activated in the presence of oxygen.

Materials and Methods

Chloroplast preparation and incubation. Intact chloroplasts were isolated from freshly-harvested spinach leaves according to the method Cockburn et al. [9] or to a modification [10] of the method described by Jensen and Bassham [11]. Wheat chloroplasts were prepared from protoplasts as described previously [8]. For spinach chloroplasts, experiments were done in a reaction medium containing 330 mM sorbitol, 50 mM Hepes (pH 7.6), 10 mM NaCl, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 0.5 mM KH_2PO_4, 10 mM glucose, 1600 units catalase/ml and substrates as indicated. Wheat chloroplasts were incubated in a medium containing 400 mM sorbital, 25 mM Hepes (pH as indicated), 10 mM EDTA, 1100 units catalase/ml, 0.2 mM KH_2PO_4 and 10 mM glucose. Reaction mixtures were made anaerobic by the addition of glucose oxidase (50 units/ml). Oxygen concentration was monitored by Hansatech [12] or other Clark-type electrodes. The system used for illumination and measuring 9-aminoacridine fluorescence was as described by Tillberg et al. [13] (Fig. 2) or Horton [14] (Fig. 9). The chlorophyll concentration was between 30 and 50 μg/ml and the temperature was 20°C.

Substrate determinations. NADP was determined by enzymic cycling [15] after terminating reactions with $HClO_4$ (final concentration 0.7 M). For determination of malate, 100 μl of chloroplast suspension were added to 900 μl water placed on a boiling-water bath. After 5 min the suspension was centrifuged and assayed for malate [16].

Measurement of enzyme activity. Enzyme activities were measured at 20°C. For measurements of fructose-bisphosphatase activity in spinach chloroplasts, samples were withdrawn from the chloroplast suspension and were added immediately to a medium containing 20 mM triethanolamine (pH 7.8), 5 mM $MgCl_2$, 2.4 mM EDTA, 1 mM fructose 1,6-bisphosphate, 0.2 mM NADP, 16 units/ml phosphoglucoisomerase and 1 unit/ml glucose-6-phosphate dehydrogenase. Fructose-bisphosphatase activity in wheat chloroplasts was measured as described previously [17]. NADP-malate dehydrogenase activity was measuring by rapidly withdrawing 50-μl aliquots of chloroplast suspension and assaying immediately in a 1-ml reaction mixture containing 0.5 mM oxaloacetate, 0.2 mM NADPH, 1 mM EDTA, 15 mM 2-mercaptoethanol, 0.05% (v/v) Triton X-100, 100 mM Tris-HCl (pH 7.8) [18]. Under these conditions, assays were linear for between 2 and 4 min. Mercaptoethanol served to stabilise the enzyme but did not itself lead to activation during the course of the assay [19].

Chlorophyll. Chlorophyll was determined by the method of Arnon [20].

Results

Fig. 1 shows the activity of stromal fructose-bisphosphatase in intact chloroplasts as a function of

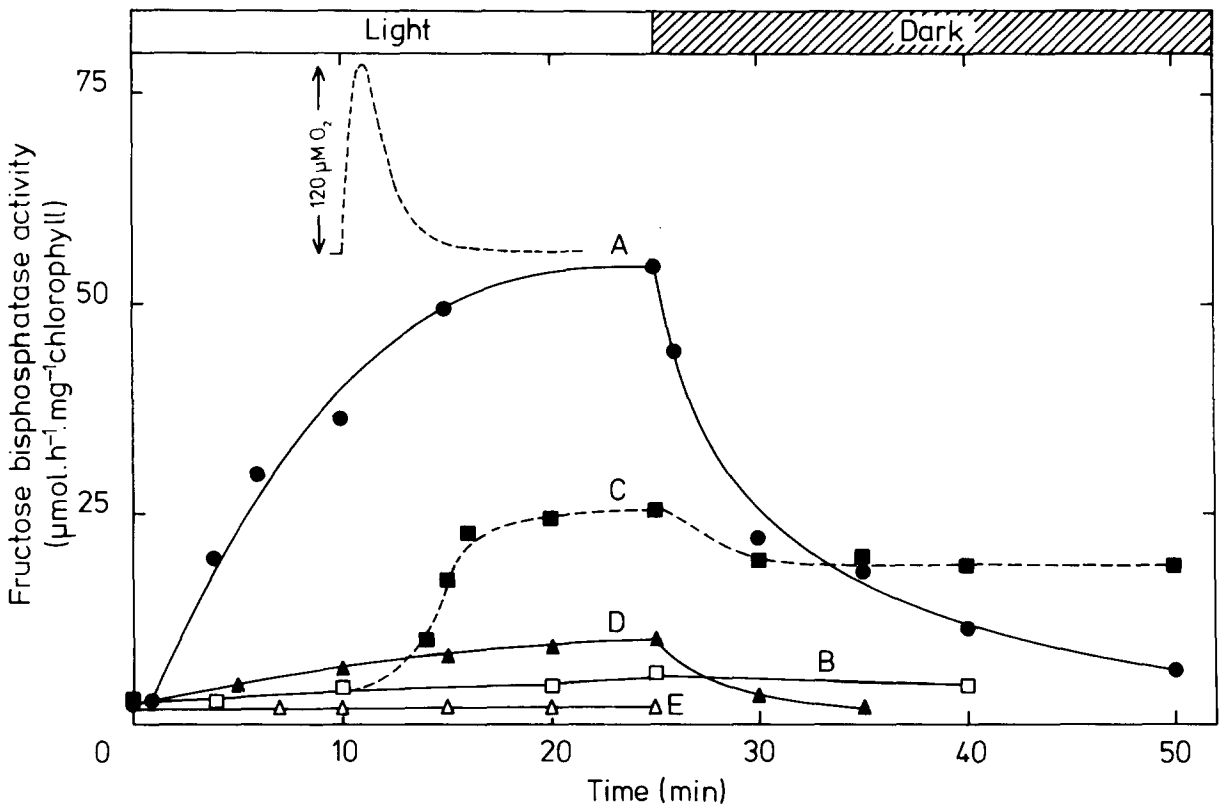


Fig. 1. Activity of fructose-bisphosphatase in intact spinach chloroplasts illuminated with 114 $W \cdot m^{-2}$ red light. (A) 1 mM HCO_3^- and approx. 300 μM O_2 (●), (B) 1 mM HCO_3^- present, anaerobic (□); (C) 1 mM HCO_3^-, initially anaerobic, but O_2 was generated after 10 min by addition of H_2O_2. After reaching a maximum concentration of 120 μM (see inset in upper left-hand corner) it was removed by the enzymic oxygen trap (■); (D) 1 mM oxaloacetate and approx. 300 μM O_2 present (▲); (E) 1 mM oxaloacetate present, anaerobic (△). Enzyme activity was measured as described in Materials and Methods.

illumination time in the presence and absence of oxygen. In the anaerobic sample, oxygen was removed by a glucose/glucose oxidase trap. Fructose-bisphosphatase was almost inactive in the dark. After a short lag, the enzyme was activated in the light in the presence of oxygen and bicarbonate (Fig. 1A) and was inactivated upon darkening. Oxaloacetate decreased light activation of the enzyme in the presence of oxygen (Fig. 1D). The extent of inhibition of light activation by oxaloacetate under aerobic conditions was dependent on light intensity. Inhibition was stronger at low than at high light intensities (cf. Ref. 17). In the absence of oxygen, light activation of fructose-bisphosphatase was minimal (Fig. 1B) and the very small activation observed was again inhibited by oxaloacetate (Fig. 1E). However, when oxygen was added to an anaerobic sample, enzyme activation occurred (Fig. 1D). Removal of oxygen inhibited further activation. Darkening in the anaerobic state failed to result in significant inactivation of previously light-activated fructose-bisphosphatase [8]. When dithiothreitol was added to darkened chloroplasts, very slow activation of fructose-bisphosphatase was observed irrespective of whether or not the chloroplasts were kept anaerobic (data not shown).

Fig. 2 shows 9-aminoacridine fluorescence quenching in a suspension of chloroplasts under different conditions. 9-Aminoacridine is a fluorescent amine which distributes, according to the Henderson-Hasselbach equation, between compartments of different acidity. When trapped in the acidified thylakoid compartment, it is non-flu-

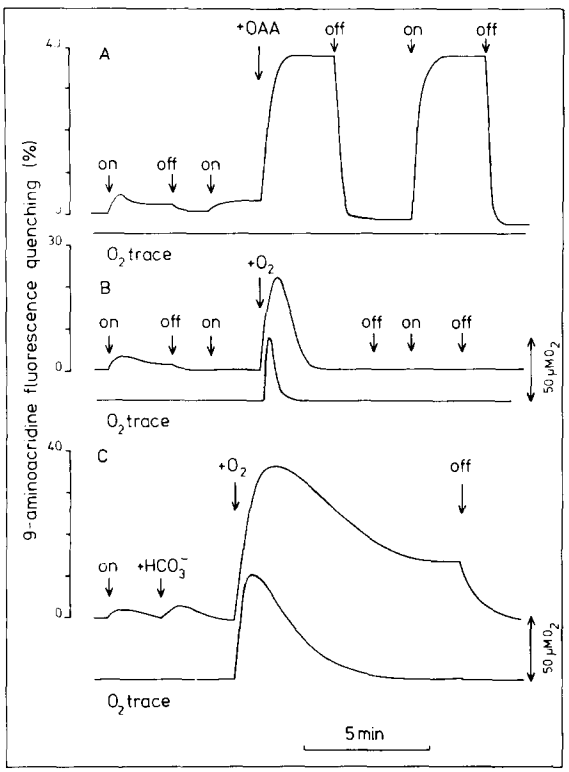


Fig. 2. 9-Aminoacridine fluorescence in a suspension of intact spinach chloroplasts. Illumination with 114 $W \cdot m^{-2}$ red light as indicated. O_2 was absent except when indicated on the O_2 trace. (A) 1 mM oxaloacetate (OAA) was added as indicated; (B) H_2O_2 was added in the absence of bicarbonate to generate O_2 as shown by O_2 trace. Note formation of a proton gradient in the presence of O_2; (C) bicarbonate (1 mM) was added in the light. Subsequent addition of H_2O_2 generated O_2 and allowed proton gradient formation. This triggered photosynthesis as indicated by the persistence of a significant proton gradient after most of the O_2 had been removed by the enzymic trap.

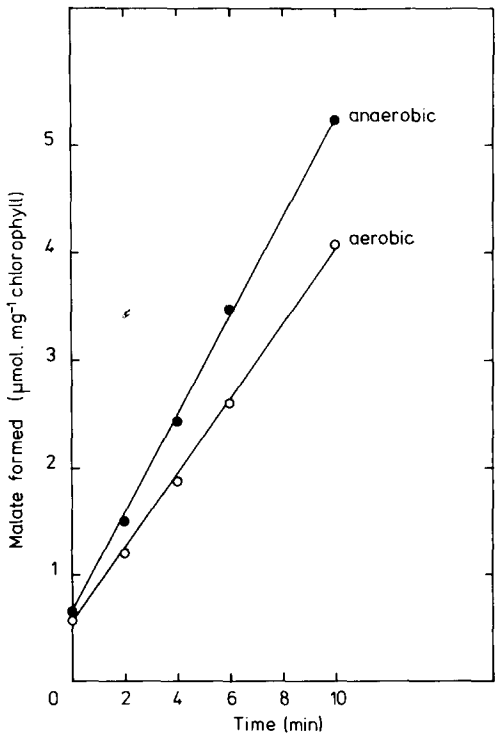


Fig. 3. Malate formation from added oxaloacetate (0.5 mM) by intact spinach chloroplasts illuminated with rate-saturating red light (330 $W \cdot m^{-2}$) in the presence (○) and in the absence (●) of approx. 300 μM O_2.

orescent. In the absence of oxygen, quenching of 9-aminoacridine fluorescence was insignificant both in the absence and in the presence of bicarbonate, indicating the absence of a large proton gradient in the chloroplasts. Under the same conditions, light activation of fructose-bisphosphatase was suppressed (Fig. 1) and photosynthetic oxygen evolution was inhibited [7]. Addition of oxaloacetate caused considerable 9-aminoacridine fluoresence quenching (Fig. 2A). Reduction of oxaloacetate to malate was actually promoted by anaerobiosis both under light saturation (Fig. 3) and under light limitation (data not shown), indicating that anaerobiosis in itself does not inhibit electron flow (see, however, Ref. 21). Electron transport to oxaloacetate facilitates proton transport across the thylakoids and generates a large proton gradient. Quenching of 9-aminoacridine fluorescence induced by oxaloacetate was similar to that produced by addition of oxygen (Fig. 2B and C) which also acts as an electron acceptor. Quenching decreased to the original level when oxygen was removed and bicarbonate was absent (Fig. 2B). In the presence of bicarbonate some quenching persisted even after the oxygen level had decreased (Fig. 2C). The residual quenching indicates maintenance of a proton gradient by electron transport to CO_2 [7]. CO_2 reduction was initiated by oxygenation of anaerobic chloroplasts and it continued even at the very low concentration of oxygen determined by photosynthetic oxygen evolution on the one hand and consump-

tion by the active enzyme trap on the other.

The finding that fructose-bisphosphatase was inactive in illuminated anaerobic chloroplasts (Fig. 1) posed the question of whether or not over-reduction could block electron transport in such a way as to leave acceptors on the reducing side of Photosystem I oxidised. This would explain the absence of light activation of fructose-bisphosphatase which is believed to be activated through reduction mediated by ferredoxin and thioredoxin [22]. However, Fig. 4 shows that chloroplast NADP levels decreased on illumination of chloroplasts under both aerobic and anaerobic conditions, indicating continued reduction of NADP and availability of electrons at the reducing side of Photosystem I. Indeed, reduction after 5 min illumination was more extensive under anaerobic conditions. Under aerobic conditions the increase in NADP seen after 1 min illumination was brought about in part by an increase in the ratio of 3-phosphoglycerate to dihydroxyacetone phosphate after a transient decrease in this ratio [23].

In contrast to fructose-bisphosphatase, NADP-malate dehydrogenase exhibited significant light activation in the presence and in the absence of oxygen (Fig. 5). Unexpectedly, the enzyme became markedly more active under anaerobiosis than at air levels of oxygen, not only at low light intensities (data not shown) when drainage of electrons to oxygen might be expected to oxidise the ferredoxin/thioredoxin system, but also at very high light intensities. Evidently lack of reductant cannot explain lack of light activation of fructose-bisphosphatase under anaerobiosis.

Previous work has shown that enzyme activation in chloroplasts proceeds only at an alkaline stromal pH [24]. Under anaerobiosis the transfer of protons into thylakoids which results in an alkalinisation of the stroma is diminished (Fig. 2). It is therefore possible that low stromal pH might be responsible for preventing light activation of

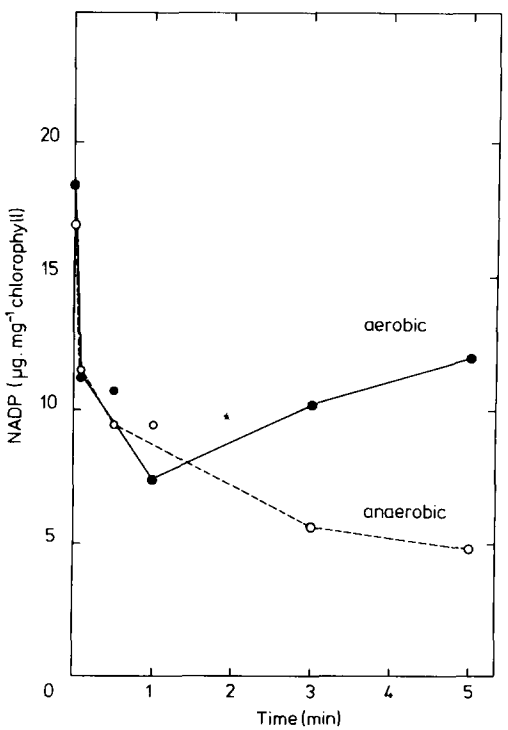


Fig. 4. NADP levels in intact spinach chloroplasts illuminated with 580 $W \cdot m^{-2}$ red light in the presence (●) and absence (○) of approx. 300 μM O_2.

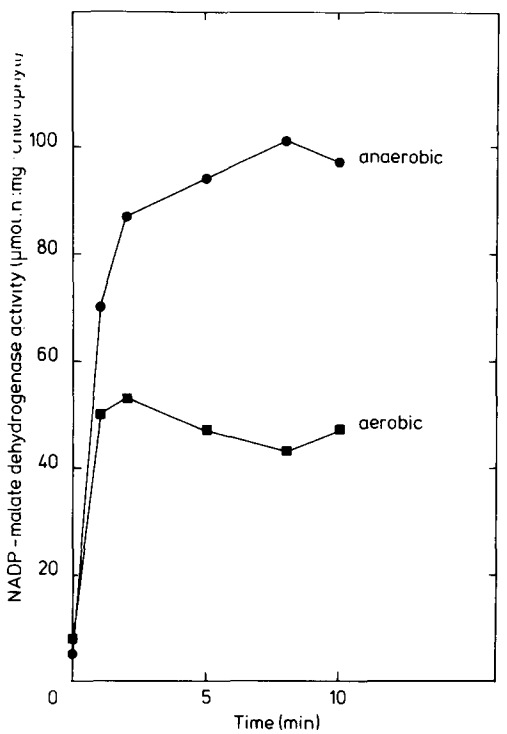


Fig. 5. Light-dependent activation of NADP-malate dehydrogenase in intact spinach chloroplasts under aerobic (approx. 300 μM O_2) or anaerobic conditions. Bicarbonate was absent. Illumination with 330 $W \cdot m^{-2}$ red light. Enzyme activity was measured as described in Materials and Methods.

fructose-bisphosphatase under anaerobiosis. When the pH of the medium was raised from pH 7.3 to pH 8.6, light activation of fructose-bisphosphatase was indeed stimulated (Fig. 6). However, this stimulation was particularly pronounced when a substrate such as dihydroxyacetone phosphate was also present. Oxaloacetate could not substitute for dihydroxyacetone phosphate. Under anaerobic conditions, oxaloacetate failed to induce much light activation of fructose-bisphosphatase (Fig. 1) although NADP-malate dehydrogenase was readily light activated (Fig. 5) and electron transport to oxaloacetate supported a large proton gradient in anaerobic chloroplasts (Fig. 2) giving rise to alkalinisation of the stroma.

In contrast to the effects of oxaloacetate and dihydroxyacetone phosphate, a low concentration of 3-phosphoglycerate increased light activation of fructose-bisphosphatase in anaerobic chloroplasts even at a more acid external pH (pH 7.6) (Fig. 7). Although data were somewhat variable, anaerobic chloroplasts illuminated at pH 7.6 usually reduce 3-phosphoglycerate, which thereby supports a moderate proton gradient [7]. The difference between the effects of 3-phosphoglycerate and dihydroxyacetone phosphate would appear to lie not in their provision of a substrate such as triose phosphate, which would be formed when 3-phosphoglycerate is reduced (fructose bisphosphate would also result from the condensation of two molecules of triose phosphate), but in the alkalinisation of the stroma which would be occasioned only by the addition of 3-phosphoglycerate. The data in Fig. 8 show the relationship between alkalinisation of the stroma and the substrate requirement for fructose-bisphosphatase activation in anaerobic wheat chloroplasts. At pH 7.6, no light activation was observed in the presence of either 3-phosphoglycerate or dihydroxyacetone phosphate. (The inability of these chloroplasts to reduce 3-phos-

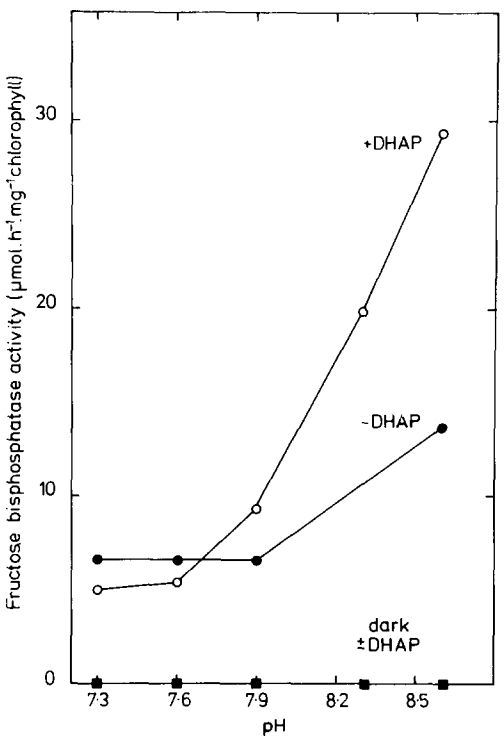


Fig. 6. Light activation of fructose-bisphosphatase in intact spinach chloroplasts measured after 5 min illumination with 114 $W \cdot m^{-2}$ red light. Effect of pH of the suspending medium and of dihydroxyacetone phosphate (1.2 mM). DHAP, dihydroxyacetone phosphate.

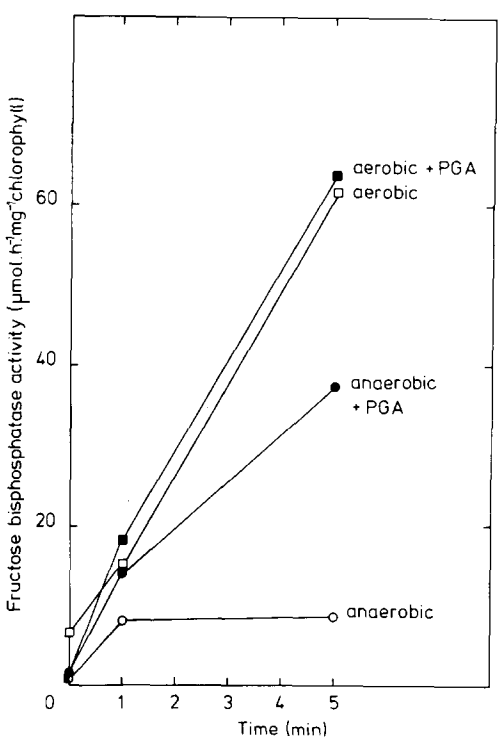


Fig. 7. Light activation of fructose bisphosphatase in intact spinach chloroplasts in the presence and absence of approx. 300 μM O_2 and 0.5 mM 3-phosphoglycerate (PGA). CO_2 was not rigorously excluded. Illumination with 580 $W \cdot m^{-2}$ red light.

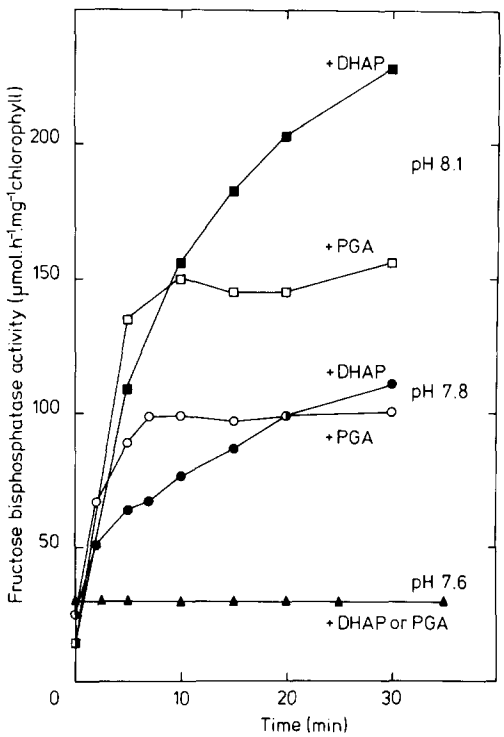


Fig. 8. Effect of 1 mM 3-phosphoglycerate (PGA) and 1 mM dihydroxyacetone phosphate (DHAP) on light activation of fructose-bisphosphatase in intact wheat chloroplasts at different pH values of an anaerobic suspending medium. Illumination was with 330 $W \cdot m^{-2}$ red light.

phoglycerate is simply a reflection of chloroplast variability mentioned above. Other anaerobic chloroplast preparations reduced 3-phosphoglycerate.) Evidently alkalinisation of the stroma was insufficient for the activation of fructose-bisphosphatase to proceed. At pH 7.8, light activation was fast in the presence of 3-phosphoglycerate and slow when dihydroxyacetone phosphate was added, reflecting more efficient alkalinisation of the stroma in the presence of 3-phosphoglycerate. However, the extent of activation was greater in the presence of dihydroxyacetone phosphate which did not intercept electrons necessary for enzyme reduction [17]. At pH 8.1, light activation was extensive in the presence of both substrates while te differences between the effects of dihydroxyacetone phosphate and 3-phosphoglycerate were even more pronounced than at pH 7.8.

Activation of fructose-bisphosphatase was shown in Fig. 1 to be prevented when oxaloacetate was the only substrate present in an anaerobic chloroplast suspension illuminated at pH 7.6. However, light activation was considerable when dihydroxyacetone phosphate was present together with oxaloacetate (data not shown), further demonstrating that stroma alkalinisation and presence of substrate are important factors in the light activation of this enzyme and that they can offset the counterbalancing effect that a diversion of electrons to a substrate such as oxaloacetate has on reductive enzyme activation [17].

The present data suggest that the inhibition of CO_2 reduction under anaerobiosis might be due to inactivity of fructose-bisphosphatase as well as a lack of ATP [7]. In the experiment shown in Fig. 9, chloroplasts were illuminated with bicarbonate in the presence of oxygen. Under these conditions, fructose-bisphosphatase would be light activated. 9-Aminoacridine fluorescence indicated formation of a large proton gradient on actinic illumination and chlorophyll fluoresence was quenched after a fast initial increase, which would be expected as a consequence of proton gradient formation and oxidation of Q [25]. After photosynthetic oxygen evolution had reached its maximum rate, the sample was made anaerobic by the addition of glucose oxidase. As a consequence of anaerobiosis, 9-aminoacridine fluorescence quenching decreased somewhat, although a significant proton gradient was maintained by substrate reduction. Surprisingly, chlorophyll fluorescence decreased. Darkening in the absence of oxygen prevented oxidative inactivation of fructose-bisphosphatase (Fig. 1 and Ref. 8). However, strong actinic illumination after some time in the weak illumination provided by the measuring beam did not result in significant quenching of 9-aminoacridine fluoresence, although again chlorophyll fluorescence decreased after a transient increase. A proton gradient was reestablished after addition of O_2, shown by 9-aminoacridine fluorescence quenching and indicating the initiation of CO_2 reduction [7]. Again, there was a surprising increase in chlorophyll fluorescence on the addition of oxygen. Another dark period again caused an inhibition of electron transport as seen by the lack of a response of 9-aminoacridine fluorescence to a third period of

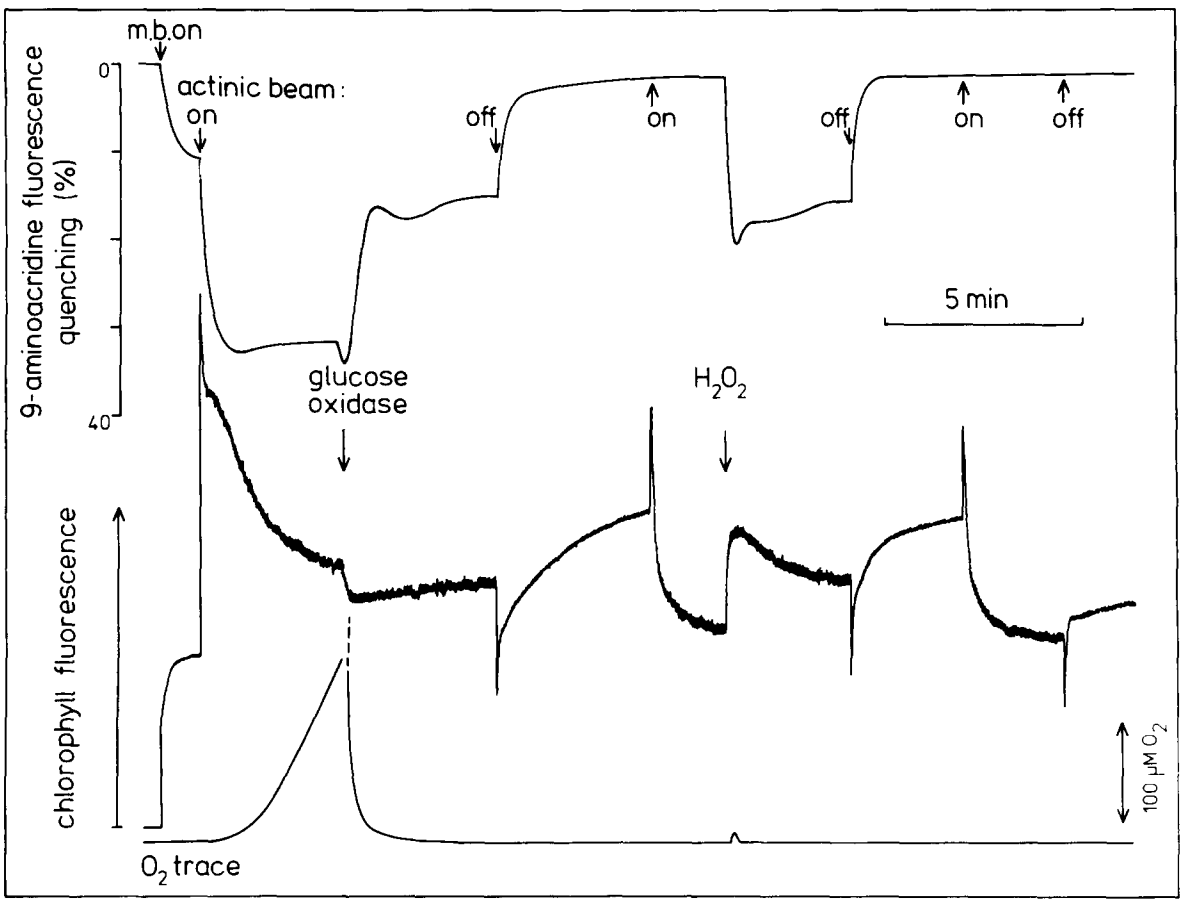


Fig. 9. Simultaneous measurement of 9-aminoacridine fluorescence (2.5 µM 9-aminoacridine), chlorophyll fluorescence and O_2 concentration in a suspension of intact spinach chloroplasts supplied with 5 mM $NaHCO_3$. The rate of CO_2-dependent O_2 evolution was 60 $\mu mol \cdot h^{-1} \cdot mg^{-1}$ chlorophyll. On the addition of glucose oxidase, the back-off voltage was switched off, shown by the break in the O_2 trace. Subsequently 2 µl of approx. 20 mM H_2O_2 were added. The measuring beam (m.b.) intensity was 0.8 $W \cdot m^{-2}$ and the actinic beam intensity 160 $W \cdot m^{-2}$ red light. The volume was 2 ml.

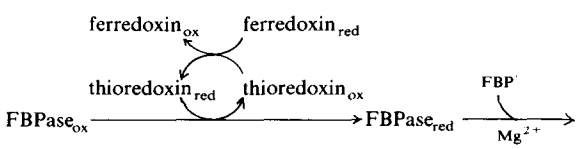
illumination, though again chlorophyll fluorescence was quenched (the changes in chlorophyll fluorescence on illumination under anaerobiosis will be the subject of a forthcoming paper). Hence, it may be concluded that inhibition of photosynthesis can be explained both by inactivity of fructose-bisphosphatase and by lack of ATP, but lack of ATP alone is sufficient to account for the observed inhibition.

Discussion

Two main points require comment; one concerning the variability of chloroplast response, the other induction in photosynthesis. Anyone who has studied photosynthesis in intact chloroplasts will have observed considerable variability between different chloroplast preparations from the same species. For example, some highly active preparations do not require the addition of catalase for maximum performance (catalase destroys H_2O_2 which arises through photosynthetic oxygen reduction [26]). Quantum requirements for oxygen evolution with CO_2 as the acceptor may be as low as 10 quanta per O_2 evolved, but very often reach much higher values [27]. The pH optimum of the assay medium used for spinach chloroplasts is often close to pH 7.6, but some (not necessarily very good) chloroplast preparations exhibit better performance at higher pH. Much of this variability

may reflect the sensitivity of the balance between oxidative enzyme inactivation and reductive activation which is mediated by the chloroplast's light activation system [28]. As mentioned in the Introduction, it is also evident that light activation of enzymes such as fructose-bisphosphatase shows a varying sensitivity to the addition or depletion of chloroplast substrates. These differences may simply reflect variations in the content of endogenous substrates or else in the capacity of the illuminated chloroplast to generate the required substrate through CO_2 fixation. For example, if no rigorous precautions are taken to ensure that endogenous substrates are depleted, light activation of enzymes may be observed in chloroplasts even in the absence of added CO_2 [17]. When such activation is observed, added substrates can only be effective if, with respect to activation, substrate saturation has not already been achieved.

The substrate requirement for fructose-bisphosphatase activation requires further explanation. Evidently, electron pressure generated during illumination of intact chloroplasts under anaerobic conditions is sufficient for reductive activation of malate dehydrogenase. Furthermore, P. Schürmann and Y. Kobayashi (unpublished results) have shown that purified fructose bisphosphatase is reduced when illuminated together with thylakoids, ferredoxin and thioredoxin under anaerobic as well as aerobic conditions. In contrast, the results presented above show that the enzyme is only poorly activated in illuminated anaerobic chloroplasts either at high external pH in the absence of substrate or at low pH in the presence of substrates. Wolosiuk et al. [29], I.E. Woodrow and D.A. Walker (unpublished results) and P. Schürmann and Y. Kobayashi (unpublished results) have suggested that fructose bisphosphate (FBP) and the reductant, thioredoxin, act sequentially to activate fructose-bisphosphatase (FBPase):



$FBPase_{red} \cdot FBP$
active enzyme-substrate complex

Moreover, Soulié et al. [30] have shown that the equilibrium of the first step, the Mg^{2+}-independent [31,32] reduction of the enzyme, lies far to the left, in favour of the oxidised form of the enzyme. Clearly, the degree to which the enzyme is reduced in chloroplasts will depend upon the interplay between the degree of enzyme reduction (i.e., electron pressure) and the extent to which reaction of the reduced enzyme with its substrate, fructose bisphosphate (an Mg^{2+}-dependent reaction [29]) shifts the overall reaction so that in spite of the unfavourable equilibrium of reduction, formation of the active enzyme is favoured. Nor in this connection should the possible influence of stromal Mg^{2+} concentration be overlooked. Wolosiuk et al. [29] have clearly shown enhancement of reductive activation by fructose bisphosphate to be dependent upon Mg^{2+} concentration. (The comments made above assume that the enzyme reduced in vivo by thioredoxin is similar to the enzyme reduced in vitro by dithiothreitol.)

We propose that electron pressure generated in intact chloroplasts even under high intensity illumination cannot bring about complete reduction of the enzyme unless this is facilitated by addition of substrate and by development of a proton gradient, the latter acting not only via the alkalinisation of the stroma, but also perhaps through the development of an Mg^{2+} concentration gradient [33]. In the presence of oxygen, stroma alkalinisation will occur in the light and slow autocatalytic substrate accumulation is possible even when very little CO_2 is available. Substrate binding to the enzyme will permit extensive enzyme activation. In the absence of oxygen, inhibition of proton gradient formation will occur and the consequent low stromal pH will decrease electron pressure on the one hand and will curtail Benson-Calvin cycle activity on the other. Low substrate levels will in turn tend to prevent the shift in equilibrium which favours reduction. In consequence, most of the enzyme will remain oxidised and inactive in the light. The concerted interplay of these factors is necessary for extensive enzyme activation, and this in turn is needed for further substrate accumulation until substrate levels are reached which support steady-state rates of photosynthesis.

The present results allow some reconciliation of

two conflicting explanations of photosynthetic induction. Lags in the onset of carbon assimilation have been attributed both to light activation of key enzymes and to autocatalytic build-up of substrates. Induction may be considerably lengthened by long periods in the dark, whereas provision of reductant by the electron-transport chain for light activation is probably a rapid process. If light-driven activation is a prerequisite which builds on autocatalysis and allows further autocatalysis to proceed, then the long induction periods which follow prolonged darkness can be accommodated in this concept much more readily than the alternative which would equate induction with enzyme activity alone. Moreover, recognition of the obvious fact that photosynthesis requires light-generated assimilatory power (in the form of ATP and NADPH) in order to proceed and that enzymes such as fructose-bisphosphatase will not readily function in the relatively acid environment offered by the darkened stroma, might diminish the tendency to emphasise a multiplicity of redox on/off switches in the Benson-Calvin cycle. In so doing attention might then be transferred to the unquestionable needs for repair and modulation. The sulphydryl groups of enzymes must be afforded protection against light-generated oxidants if photosynthesis is to continue unabated. Similarly, there is a growing awareness that a multi-enzymic, cyclic sequence must be adequately modulated if the competing requirements of individual steps are to be kept in balance and if the regenerative aspects of the cycle are neither to exceed nor fall below the need for export of metabolites from the stroma to the cytosol. The complexity of factors which, it is now clear, affect the activity of fructose-bisphosphatase seem admirably suited to modulation. The act that NADP-malate dehydrogenase, for example, requires reductive activation alone might well underline the fact that differences in activation requirements could point to major differences in regulatory action.

Acknowledgements

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