Biochimica et Biophysica Acta 849 (1986) 244–253
Elsevier

BBA 41981

Factors affecting photosynthetic induction in spinach leaves

Roslyn T. Prinsley * and Richard C. Leegood **

Research Institute for Photosynthesis, Department of Botany, University of Sheffield, Sheffield, S10 2TN (U.K.)

(Received August 21st, 1985)
(Revised manuscript received January 6th, 1986)

Key words: Photosynthesis; Benson-Calvin cycle; Induction; Carbon assimilation; (Spinach leaf)

Induction of photosynthetic carbon assimilation in spinach leaves in saturating CO_2 has been studied in relation to such factors as the length of the preceding dark period, intensity of pre-illumination, irradiance and the phosphate status of the leaf, through measurements of O_2 evolution and of changes in leaf metabolites. (i) In low light, induction comprised two phases, an oxygen burst and a slow rise to the steady-state rate. Both the oxygen burst and induction (measured as induction gain or loss) correlated with the amount of glycerate-3-P present in the leaf prior to illumination. (ii) The induction period in dark-adapted leaves was dependent on the irradiance, being greater at higher irradiance. In the early stages of induction at different irradiances metabolite levels rather than the rate of electron transport appeared to limit the rate of photosynthesis. (iii) The induction period was extended in leaves from plants grown in low phosphate or where phosphate was sequestered by the use of 2-deoxyglucose while the induction period was shortened by feeding phosphate to phosphate-deficient leaves. Phosphate deficiency was characterized by low glycerate-3-P in the dark and a slow build-up to lower steady-state levels of metabolites in the light, with the absence of characteristic peaks in the contents of glycerate-3-P, Fru-1,6-P_2 and the ATP/ADP ratio during induction. These observations serve to emphasise that the conversion of existing metabolite pools and metabolite build-up are important determinants of the induction period in leaves.

Introduction

Our knowledge of regulatory mechanisms underlying induction phenomena in photosynthetic carbon assimilation [1,2] is almost entirely based upon work done with isolated chloroplasts. Considerable evidence has been obtained that emphasises the importance of metabolite build-up during induction. Thus catalytic amounts of Calvin cycle intermediates which penetrate the chloroplast envelope dramatically shorten the induction period [3,4]. Conversely, a high concentration of orthophosphate in the medium surrounding isolated chloroplasts results in enforced export of triose phosphate, which impedes autocatalysis and extends the induction period. If P_i in the medium is low, the rate of photosynthetic carbon assimilation decreases substantially after a very short time, since it is supported only by the comparatively slow rate of P_i release during starch synthesis [5]. Isolated chloroplasts therefore exhibit a distinct, rather sharp, P_i optimum at which sufficient P_i is important into the chloroplast to support photophosphorylation, yet external P_i is not so high as to lead to excessive export of triose phosphate.

* Present address: Commonwealth Science Council Secretariat, Marlborough House, Pall Mall, London, SW1Y 5HX, U.K.
** To whom correspondence should be addressed.

Abbreviations: P_i, inorganic phosphate; Rbu-1,5-P_2, ribulose-1,5-bisphosphate.

As far as induction in leaves is concerned, these observations imply both that the level of intermediates in the darkened chloroplast and the concentration of cytosolic P_i will be crucial determinants of the length of the induction period. However, our understanding of the process of induction in leaves is largely restricted to comparison with isolated chloroplasts. Thus while induction in leaves is shortened by pre-illumination, just as it is in isolated chloroplasts, there is little direct evidence that the underlying events in leaves and chloroplasts are the same and there is good reason to suspect that there may be major differences. For example, the ready availability of phosphate in the suspending medium of isolated chloroplasts means that the environment of the isolated chloroplast differs radically from the cytosol, in which the supply of phosphate depends upon the continued synthesis of sucrose. We have investigated induction in spinach leaves in relation to both irradiance and P_i status (the latter manipulated by growing plants in the absence of phosphate or by supplying leaf discs with phosphate-sequestering agents), and through simultaneous measurements of the rate of carbon assimilation and of changes in photosynthetic metabolites.

Materials and Methods

Materials

Spinacia oleracea L. (Yates hybrid 102) was grown in hydroponic culture in a greenhouse with supplemental lighting. For gas-exchange experiments, leaf discs of 10 cm^2 were used. For metabolite measurements, three discs of 2.5 cm^2 were used for each point; all leaf discs for a single experiment derived from one large leaf. $NaH^{14}CO_3$ was obtained from Amersham International (U.K.). Rbu-1,5-P_2 was obtained from Sigma Chemical Company (Poole, U.K.). All other biochemicals were from Boehringer (Mannheim, F.R.G.).

Measurement of gas exchange

O_2 evolution was measured as described by Delieu and Walker [6]. The air in the chamber contained approx. 5% CO_2 and the temperature was 20°C. Illumination was provided by a 150 W tungsten/halogen source (Volpi Intralux 5000) filtered through heat-absorbing glass, a heat-reflecting interference filter and either a Corning 4-96 glass filter (blue light) or Schott RG 610 and Balzers K65 filters (red light).

Pretreatment of leaf discs

Leaf discs from opposite sides of the midrib were floated on sugar solutions or on water in the dark for a period of 2–3 h. For treatment with P_i, leaf discs were floated on 20 mM potassium phopshate at pH 7 for 1 h in white light (150 $W \cdot m^{-2}$). They were then darkened for 3 h before measurements were made.

Preparation of leaf extracts

Leaf discs were placed in a cylindrical chamber (5 cm high and 10 cm diameter) which was sealed at the top and the bottom with cling film held in place by rubber bands. Gas was humidified and passed through the chamber. Side-illumination was provided by a projector (150 W, white) connected via a fibre-optic with two branches. At appropriate points during the time-course, leaf metabolism was stopped by freeze-clamping the leaf through the cling-film windows of the chamber.

A pellet of 1M frozen $HClO_4$ (500 μl) was ground together with the frozen leaf material in a pre-cooled pestle and mortar. The mixture was allowed to thaw and the pestle and mortar were washed with three 150 μl aliquots of 0.1 M $HCl)_4$ and centrifuged for 2 min at $2000 \times g$. The extracts were then neutralized (to pH 7) either with 5 M K_2CO_3 or with CO_2-free KOH (if used for Rbu-1,5-P_2 determination). 10 mg charcoal was added as a suspension (except where adenylates were to be measured) and the neutralized samples were centrifuged for 2 min at $2000 \times g$.

Metabolite assays

All metabolites were measured spectrophotometrically as described by Lowry and Passonneau [7]. Rbu-1,5-P_2 was determined by $^{14}CO_2$ incorporation into glycerate-3-P in the presence of Rbu-1,5-P_2 carboxylase. The assay contained 50 mm Tris-HCl/20 mM $MgCl_2$/2 mM EDTA/3 mM dithiothreitol. Assays were run in 300 μl total volume, including 12 mM $NaH^{14}CO_3$ (2 Ci ·

mol^{-1})/0.2 U Rbu-1,5-P_2 carboxylase/100 µl sample. The reaction was terminated by adding 100 µl 5 M HCl. Rbu-1,5-P_2 carboxylase was prepared as in Ref. 8. ATP and ADP were assayed using luciferin-luciferase [9]. The metabolite data presented are representative of between three and seven separate experiments.

Results and Discussion

Throughout, the experiments were done in saturating CO_2 to obviate a stomatal contribution to induction, especially in conditions where phosphate was manipulated [10] and to eliminate complications introduced by varying degrees of photorespiratory phosphate-recycling.

Effect of pre-treatment on induction

The influence of the preceding dark period and of pre-illumination on induction was studied at a low irradiance because at a high irradiance the kinetics were complex due to oscillations in the rate of oxygen evolution. In low light (5 $W \cdot m^{-2}$) the induction period in spinach leaf discs consisted of two phases (Fig. 1a): (i) a burst in the rate of oxygen evolution with a peak at about 30 s, which was followed by (ii) a gradual increase in the rate of O_2 evolution until a steady state was reached.

Following pre-illumination at 5 $W \cdot m^{-2}$, leaf discs were left for between 1 and 15 min in the dark. The length of the subsequent induction period (measured as the time taken to reach a steady-state rate) increased rapidly to about 3 min with increasing dark period (Fig. 1a). From 15 min dark to 5 h dark pre-treatment, the induction period increased gradually to an average value of 6 min, but between 5 h and 15 h dark pre-treatment, there was no increase in induction period. The size of the oxygen burst decreased with an increasing dark pre-treatment.

Induction was also studied at 5 $W \cdot m^{-2}$ after 10 min in the dark, prior to which leaf discs had been pre-illuminated for 30 min (Fig. 1b). There was an increase in the oxygen burst with increasing intensities of pre-illumination. However, the presence of this burst complicated a simple assessment of induction (based on the time taken to reach a steady-state rate of oxygen evolution) in

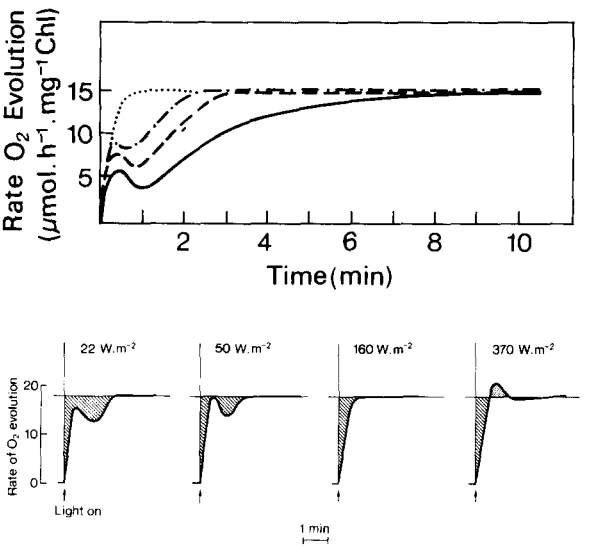

Fig. 1. (a) Rate of O_2 evolution of leaf discs at 5 $W \cdot m^{-2}$ (blue light) after various dark periods. 1 min dark (· · · · · ·); 15 min dark (· – · – ·); 1 h dark (- - - - - -); 5 h and 15 h dark, which were identical (———). (b) The effect of pre-illumination (1 h) of leaf discs at various irradiances on the induction period at 5 $W \cdot m^{-2}$ (blue light) after a dark period of 10 min. Irradiances shown are those of pre-illumination. The arrow indicates when the light was turned on. Hatched areas indicate induction loss, stippled areas indicate induction gain.

situations where the rate of O_2 evolution during the burst was greater than during the steady-state. In these instances it is more useful to think of induction in terms of McAlister's concept of induction loss (or gain) [1,11]. Induction loss is the difference between the amount of oxygen that would have been evolved had there been no induction and the amount of oxygen that was actually evolved. Thus it can be seen that there was a decreased induction loss with an increased intensity of pre-illumination (Fig. 1b).

The origin of the O_2 burst in intact chloroplasts or in leaves is unknown. In the reconstituted chloroplast system it reflects the fast rate of electron transport to $NADP^+$ [12]. Darkened spinach chloroplasts contain about 10 nmol $NADP^+ \cdot mg^{-1}$ chlorophyll [13]. On the other hand, Clayton [14] suggests that electrons accepted by quinones of the electron transport chain are responsible for the burst. The pool of quinone acceptors amounts to about 15 $nmol \cdot mg^{-1}$ chlorophyll [15]. The ferredoxin pool in the dark in spinach chloroplasts is

about 20 nmol · mg^{-1} chlorophyll [16]. The total oxygen burst deriving from the reduction of the pools of these and other electron transport intermediates would therefore be less than 20 nmol O_2. This total falls short of the measured burst, which was between 60 and 100 nmol O_2. Further evidence that electron transport components alone are not responsible for the oxygen burst is provided by the observation that there is a CO_2 'gulp' which is clearly related to the O_2 burst and which indicates that the O_2 burst is associated with Calvin cycle activity [17].

Franck [18] considered that transient bursts and gulps were manifestations of the photochemical reduction of intermediates accumulated during the dark period and available for reduction during the first minute of illumination. The O_2 burst might reflect immediate reduction of glycerate-3-P present in the stroma upon illumination, with the Rbu-1,5-P_2 formed from triose P being carboxylated, generating a CO_2 gulp. For example, Heber and Santarius [19] showed that the glycerate-3-P content of the chloroplast compartment of spinach leaves decreased rapidly upon illumination. In spinach protoplasts, chloroplastic glycerate-3-P persists in the dark, whereas other

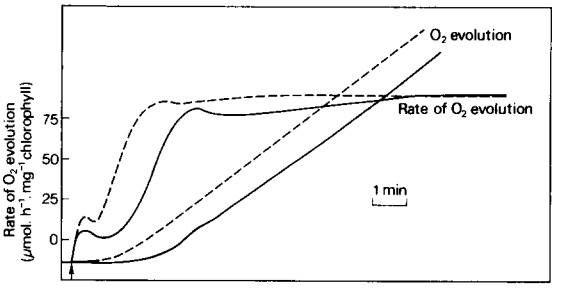

Fig. 3. The effect of feeding glucose (100 mM) to leaf discs in the dark on the rate of O_2 evolution during induction at 40 W · m^{-2}. Floated on water in dark for 6 h (———); floated on 100 mM glucose in dark for 6 h (- - - - -).

metabolites decrease rapidly [20]. The content of glycerate-3-P during steady-state carbon assimilation in illuminated leaves is also dependent on the irradiance [21]. The amount of glycerate-3-P in leaf discs was therefore manipulated by varying the length of the dark period and by varying the intensity of pre-illumination. The amount of glycerate-3-P was measured in the dark and the extent of the induction loss determined. Fig. 2 shows that there was a strong inverse correlation between the amount of glycerate-3-P in the dark

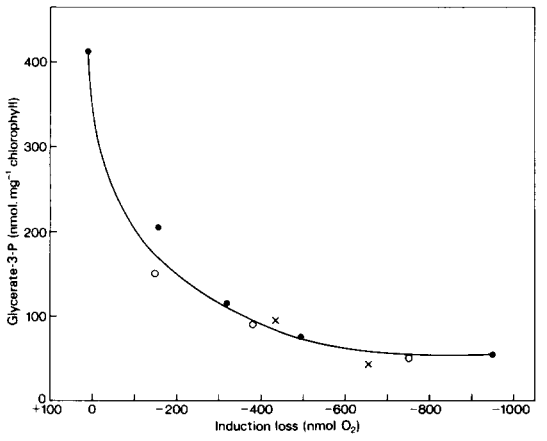

Fig. 2. The variation of glycerate-3-P levels in the dark and subsequent induction loss with varying lengths of dark period (●), with varying intensities of pre-illumination (○) or after pre-treatment with glucose (×). The induction loss was determined at 40 W · m^{-2}. From left to right on the graph, increasing lengths of dark period were 1 min, 5 min, 10 min, 15 min and 5 h; intensities of pre-illumination were 400, 180 and 20 W · m^{-2}.

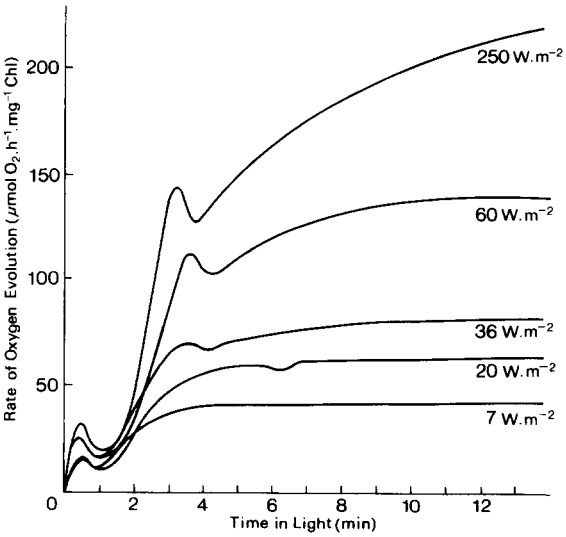

Fig. 4. The effect of irradiance (red light) on the induction period of leaf discs (taken from one leaf) which were dark-adapted for 5 h. (Note: the increase in the O_2 burst apparent with higher irradiance is a transient artefact caused by an increase in temperature within the leaf chamber.)

and the extent of the induction loss. After one minute dark there was often an induction 'gain' due to the large O_2 burst. When this induction gain occurred, it was associated with a glycerate-3-P content in the dark of at least 100 nmol · mg^{-1} chlorophyll more than during the steady state in the light. In addition, Stitt et al. [22] have shown that floating leaf discs in the dark on a solution of glucose leads to an increase in hexose phosphate levels. The amount of glycerate-3-P in the leaf

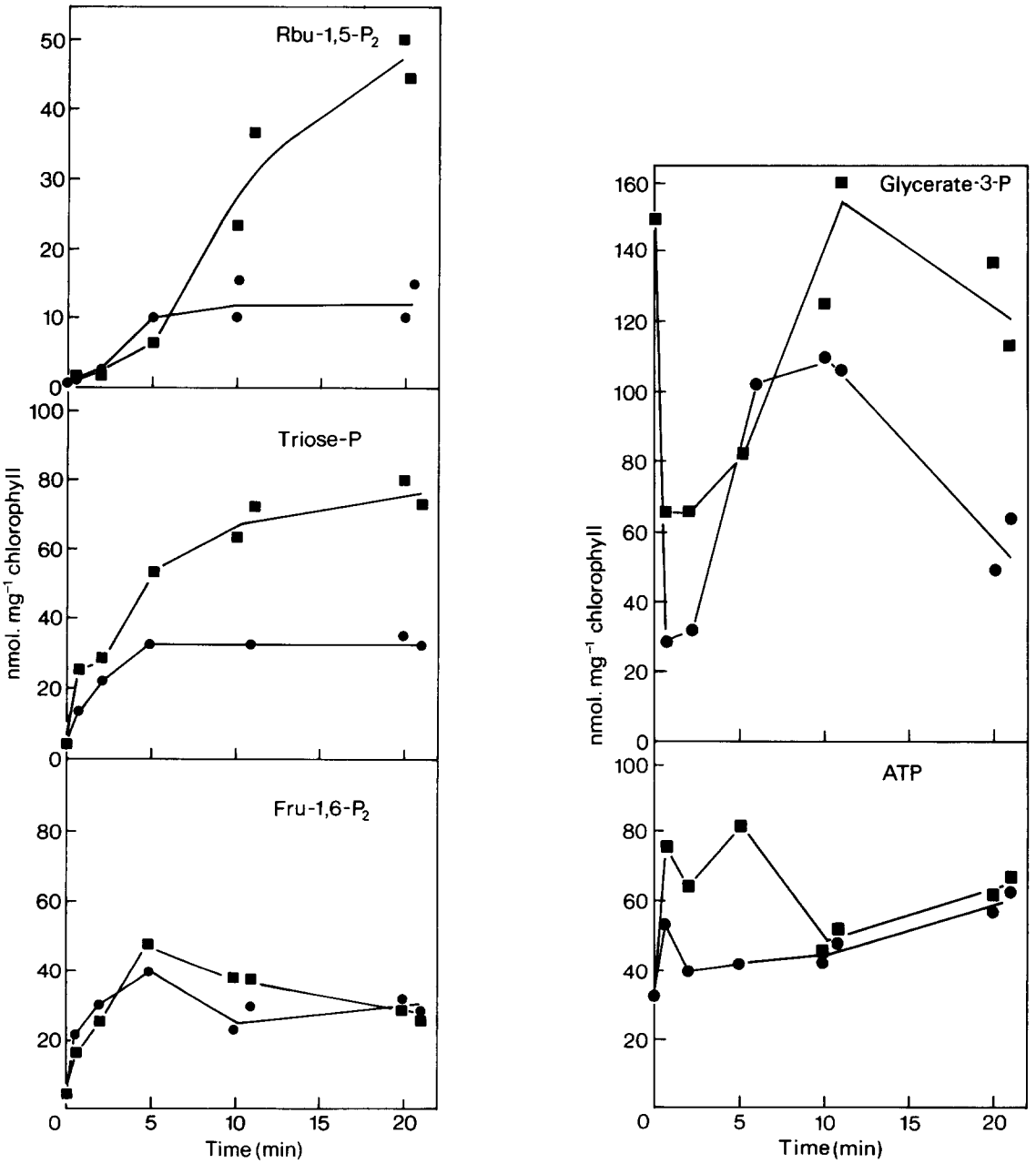

Fig. 5. Changes in levels of leaf metabolites during induction at high (■, 150 W·m^{-2}) and low (●, 15 W·m^{-2}) irradiance (white light).

discs also more than doubled after floating on 100 mM glucose for a period of 6 h in the dark and the induction loss was much reduced (e.g., an increase in the content of glycerate-3-P from 42 to 95 nmol per mg chlorophyll and a decrease in the induction loss from 655 to 435 nmol O_2, Fig. 2). Induction was not only shorter, but the O_2 burst was much larger in glucose-fed leaves (Fig. 3). The above evidence is therefore consistent with the view that immediate reduction of glycerate-3-P accounts for the oxygen burst and also primes the Calvin cycle, thus reducing the time needed for metabolite build-up and thereby shortening induction.

The effect of irradiance on induction

The time taken to reach a steady-state rate of O_2 evolution in dark-adapted leaves was influenced by irradiance such that the lower the irradiance, the shorter the induction period (Fig. 4). At higher irradiance, a second, and much larger, burst in the rate of oxygen evolution usually followed the first burst and a further increase to steady state then followed. This secondary burst was absent at very low irradiance. In this instance there was no complication by oscillations in rate because the leaves were fully dark-adapted. The time taken to reach the steady-state rate of oxygen evolution was found to be apparently proportional to the final rate of photosynthesis with respect to irradiance.

The amounts of metabolites were measured both in low light (15 $W \cdot m^{-2}$) and in high light (150 $W \cdot m^{-2}$) during induction (Fig. 5). The levels of Rbu-1,5-P_2 and triose phosphate increased with the rate of oxygen evolution. The rate of build-up of Rbu-1,5-P_2 was very similar at both high and low irradiance for the first few minutes of induction. After the first few minutes, Rbu-1,5-P_2 levels reached a steady-state at low irradiance, whereas at high irradiance, the build-up of Rbu-1,5-P_2 continued. The level of glycerate-3-P decreased initially, coinciding with the O_2 burst. The amount of ATP increased at the time of the O_2 burst (see also Refs. 20, 23 and 24). However, after this peak, ATP fell to a similar value at both high and low irradiance. Heber et al. [36] have observed similar behaviour of the ATP/ADP ratio in spinach protoplasts illuminated in high and low light.

The levels of Fru-1,6-P_2 and hexose phosphates (the latter not shown) reached a peak after about 5 min in the light, and then decreased (see Fig. 5). The changes in levels of Fru-1,6-P_2 at both high and low irradiance were very similar. The peak in Fru-1,6-P_2 indicates that Fru-1,6-P_2-ase activity limits the flux through the Calvin cycle in the early stages of induction at high and low irradiance (see, e.g. Ref. 20). The low level of Rbu-1,5-P_2 (10 $nmol \cdot mg^{-1}$ chlorophyll, equivalent to a concentration of 0.4 mM in the stroma) at this time shows that the rate of CO_2 fixation was limited by the rate of Rbu-1,5-P_2 regeneration. However, during the first 2 min in the light, the rates of photosynthesis and Rbu-1,5-P_2 levels at the two irradiances were very similar to one another, even though the rate of photosynthesis would be expected to be higher at a high irradiance due to the increased rate of electron transport. The amount of glycerate-3-P actually fell more in low light and the glycerate-3-P/triose phosphate ratio was slightly lower in low light during the first min of induction (thus the quotient $ATP \cdot H^+ \cdot NAD(P)H/ADP \cdot P_i \cdot NAD(P)^+$ would have been similar in high and in low light, compare Ref. 36, where the induction period in spinach protoplasts was much shorter). These observations indicate that the limitation on carbon assimilation at this early stage of induction was related to the availability of appropriate pools of Calvin cycle intermediates rather than to a limitation at the level of electron transport.

Thus although there was a difference between the potential rate at the two different irradiances, this potential could not be realized initially during induction because the amount of Rbu-1,5-P_2 available for carboxylation and the amount of glycerate-3-P available for reduction were too low. As the concentration of glycerate-3-P increased, the increased assimilatory power at the high irradiance could begin to take effect, and the rate of photosynthesis rapidly increased at a high irradiance. However, at the low irradiance, the rate of photosynthesis and levels of Rbu-1,5-P_2 had almost reached their steady-state levels when other constraints such as enzyme activation were removed, and therefore the induction period was almost complete by this time. These results are all consistent with the view that increased metabolite

pools are required for maximum rates of O_2 evolution, but clearly modulation of enzyme activity must also be a factor involved in regulation of photosynthesis during the early stages of induction.

Previous studies have shown that induction in isolated chloroplasts and leaves is largely independent of irradiance [1,2,11,25–28], although in leaves the situation is certainly more complex, with induction dependent on irradiance at very low light intensities [11]. Under the quite different conditions employed here (high CO_2, long dark period), induction was clearly dependent on the irradiance. The independence of induction on irradiance is not therefore an immutable property of the system and appears to depend upon the length of the preceding dark period [29]. The extent to which metabolites decrease in the dark will also depend upon the plant material and the conditions. While these factors may influence the dependence of induction on irradiance, the distinction that Rabinowitch [1] made between 'long' and 'short' induction should also be kept in mind. 'Short' induction, following brief periods of darkness, is less likely to depend upon factors such as product synthesis or long-term changes in phosphate status, and such induction is accordingly often simple and independent of irradiance.

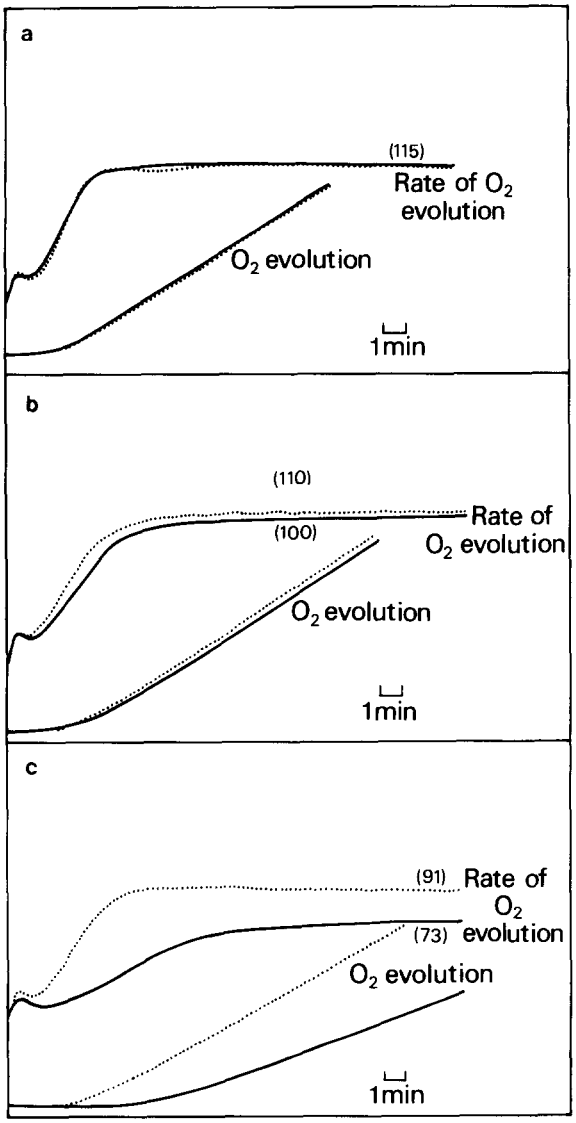

Fig. 6. The effect of feeding 25 mM P_i to spinach leaf discs on the amount of O_2 evolved and the rate of O_2 evolution during induction (illumination at 54 $W \cdot m^{-2}$, blue light). Spinach was grown in nutrient medium containing (a) 25 mM P_i (b) 1 mM P_i (c) zero P_i. Control (———), fed P_i (· · · · · ·). Rates of O_2 evolution (in μmol/h per mg chlorophyll) are shown in parenthesis.

The role of phosphate during induction in leaves

Leaves were taken from plants grown in regimes of high (25 mM), normal (1 mM) and zero phosphate present in the hydroponic nutrient medium in order to test the effect of P_i-depletion on induction in photosynthesis. Fig. 6 shows that the induction period in the leaves from plants grown in 25 mM P_i (Fig. 6a) was slightly shorter than that of the leaves from plants grown in 1 mM

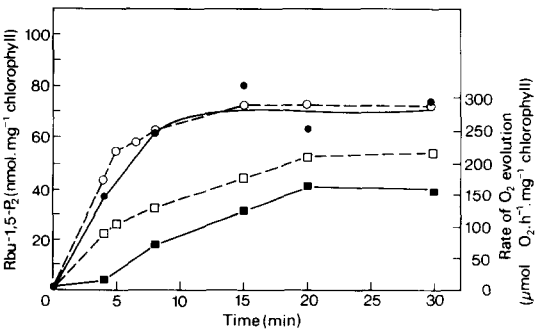

Fig. 7. The effect of feeding 25 mM 2-deoxyglucose on the rate of O_2 evolution and levels of Rbu-1,5-P_2 during induction in leaf discs from P_i-sufficient plants (illumination at 145 $\cdot m^{-2}$, white light). Rbu-1,5-P_2, control (●), fed 2-deoxyglucose (■); rate of O_2 evolution, control (○), fed 2-deoxyglucose (□).

P_i (Fig. 6b). However, the induction period of the leaves from plants grown without P_i (Fig. 6c) was twice as long as the induction period of the leaves from plants grown in 1 mM P_i.

Foyer and Spencer [30] have shown that there is a depletion of the cytosolic pol of P_i in leaves from plants grown without phosphate. If the lengthening of the induction period and decrease in rate are due to the shortage of P_i as a reactant in carbon assimilation, then feeding P_i to P_i-deficient leaves should reverse these effects. When P_i was fed to the leaf discs, it had no discernible effect on either the length of the induction period or the steady-state rate of O_2 evolution in leaves from plants grown with 25 mM P_i (Fig. 6a), and it had little or no effect on the induction period or the steady-state rate of photosynthetic carbon assimilation in leaves from plants grown with 1 mM P_i (Fig. 6b). However, when P_i was fed to leaf discs taken from plants grown in the absence of P_i, there was a shortening of the induction period by 50% and an increase in the rate of O_2 evolution by 20%, and both the induction period and steady-state rate were similar to those of the control leaves (Fig. 6c). When 25 mM 2-deoxy-D-glucose was fed in the dark to leaf discs taken from plants grown on 1 mM P_i, there was a 60% increase in the subsequent induction period and a 25% decrease in the steady-state rate of O_2 evolution (results not shown). Similar experiments were also done comparing D-mannose (which sequesters P_i as mannose-6-P) and L-mannose (which does not react with hexokinase) used as a control. The results obtained with D-mannose were similar to those were 2-deoxyglucose was used, but L-mannose was without effect.

Amounts of metabolites were measured during induction in phosphate-deficient leaves. The results obtained were very similar whether phosphate deficiency was induced by growing plants in the absence of phosphate or by treating leaf discs with agents such as 2-deoxyglucose. Fig. 7 shows simultaneous build-up of the amount of Rbu-1,5-P_2 with the rate of O_2 evolution, whether in the control leaves or in leaves depleted of phosphate by the use of 2-deoxyglucose. The rate of build-up of Rbu-1,5-P_2 in the phosphate-deficient leaf discs was therefore slower. In leaves fed 2-deoxyglucose (Fig. 7) or in leaves from plants grown in the absence of phosphate (data not shown), the amount of Rbu-1,5-P_2 was reduced to 30–50% of the steady-state levels compared to the control or P_i-fed leaf discs and was reduced more than the rate of O_2 evolution, possibly indicating limitation by the activity of Rbu-1,5-P_2 carboxylase under these conditions.

In P_i-deficient leaves, Fru-1,6-P_2 levels did not

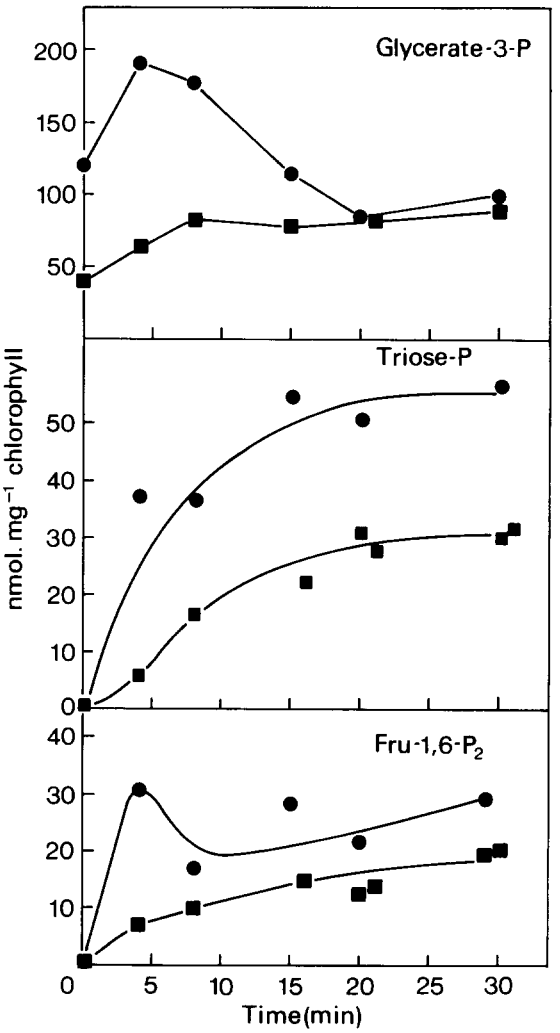

Fig. 8. The effect of feeding 25 mM 2-deoxyglucose on the levels of metabolites during incubation in leaf discs from P_i-sufficient plants (illumination at 145 $W \cdot m^{-2}$, white light). Control (●), fed 2-deoxyglucose (■). Note the difference in sampling times from Fig. 5. The data was obtained from leaf discs cut from single leaf, and it should be noted that there is considerable variation in contents and kinetics of metabolite changes between different leaves.

reach a peak during the first few minutes of induction as they did in control leaves (Fig. 8). Instead, Fru-1,6-P_2 increased in parallel with the rate of O_2 evolution. In addition, the steady-state levels of Fru-1,6-P_2 were lower in phosphate-deficient leaves. The amount of glycerate-3-P in the dark in P_i-deficient leaves decreased by 50–70% compared to darkened control leaves. The peak of glycerate-3-P seen during induction in control leaves was much reduced in P_i-deficient leaves (Fig. 8). The same was true for the ATP/ADP ratio in P_i-deficient leaves, which showed almost no change throughout induction in leaf discs fed 2-deoxyglucose. The ATP/ADP ratio was also much lower in leaves from plants grown in low P_i compared with P_i-deficient leaves fed P_i (Fig. 9).

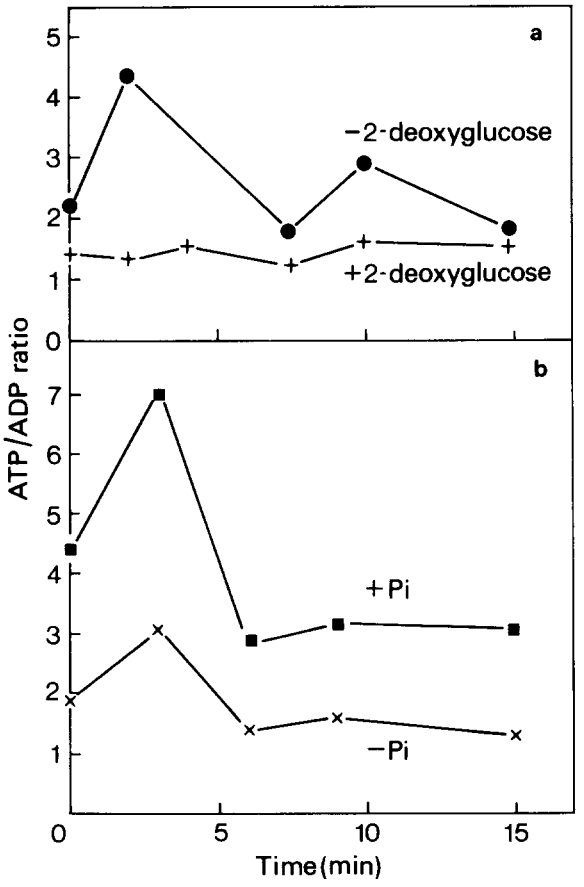

Fig. 9. Effect of phosphate status on the ATP/ADP ratio during induction in spinach leaf discs. (a) After feeding 25 mM 2-deoxyglucose to P_i-sufficient leaf discs (illumination at 145 $W \cdot m^{-2}$, white light). Control (●), fed 2-deoxyglucose (+); (b) after feeding 25 mM P_i to leaf discs from plants grown in the absence of phosphate (illumination in 5% CO_2 at 100 $W \cdot m^{-2}$). Control (×), fed P_i (■).

A simplistic approach to the effect of P_i-depletion on O_2 evolution in leaves would be to draw upon the results obtained when P_i concentration is lowered in the medium surrounding isolated chloroplasts. In isolated chloroplasts, the induction period is shortened because export of triose phosphate is diminished and the autocatalytic production of Rbu-1,5-P_2 is enhanced. Clearly, this situation does not obtain in vivo, because the induction period was increased when the leaves were depleted of P_i. This difference could be due to various factors. The first factor is that the content of glycerate-3-P was much lower in P_i-depleted leaf discs in the dark than in control leaves (see also Ref. 31). The second factor was the influence of phosphate on the adenylate status of the leaf. Although it was concluded above that the rate of O_2 evolution during the induction period did not appear to be limited by electron transport during the first few minutes at different irradiances, in the case of P_i-depleted leaves the ATP/ADP ratio was much lower and that it did not increase to any great extent at any time during the induction period. The decrease in the ATP/ADP ratio was not due to an increase in ADP levels, which remained the same in both P_i-depleted and P_i-fed leaf discs (results not shown).

The mechanism by which P_i-limitation occurs remains unclear, but low stromal P_i could affect the rate [32] or the efficiency of photophosphorylation [33]. Although a low ATP/ADP ratio is not in itself evidence either of a limitation on the rate of photophosphorylation or of poor photosynthetic performance [36], the combination of low concentrations of glycerate-3-P and a low ATP/ADP ratio would contribute to a slowing of the conversion of glycerate-3-P to glycerate-1,3-P_2 [34,35], and therefore a decline in the rate of O_2 evolution and Rbu-1,5-P_2 regeneration. Thus the lack of a primer – a large initial pool of carbon from which to build-up other intermediates – and the lack of ATP to work on the small pool present in P_i-deficient leaves means that the rate of build-up of intermediates is very slow, accounting for a long induction period while Rbu-1,5-P_2 assumes its final steady-state level.

Acknowledgements

This work was supported by the Agricultural and Food Research Council, U.K. and by the Science and Engineering Research Council, U.K. We thank David Walker, at whose suggestion this work was carried out, and Bob Heath for their criticism and encouragement and Peter Horton and Mark Stitt for helpful discussion.

References

1 Rabinowitch, E.I. (1956) Photosynthesis and Related Processes, Vol. II, pp. 1313–1432, Wiley Interscience, New York
2 Walker, D.A. (1973) New Phytol. 72, 209–235
3 Bucke, C., Walker, D.A. and Baldry, C.W. (1966) Biochem. J. 101, 636–41
4 Leegood, R.C. and Walker, D.A. (1980) Arch. Biochem. Biophys. 200, 575–582
5 Cockburn, W.A., Walker, D.A. and Baldry, C.W. (1968) Biochem. J. 107, 89–95
6 Delieu, T.J. and Walker, D.A. (1983) Plant Physiol. 73, 542–549
7 Lowry, O.H. and Passonneau, J.V. (1972) A Flexible System of Enzymatic Analysis, Academic Press, New York
8 Dietz, K.-J. and Heber, U. (1984) Biochim. Biophys. Acta. 7678, 432–443
9 Carver, K.A. and Walker, D.A. (1983) Sci. Tools 30, 1–5
10 Harris, G.C., Cheesbrough, J.K. and Walker, D.A. (1983) Plant Physiol. 71, 102–107
11 McAlister, E.D. (1937) Smithsonian Misc. Coll., 95, 1–17
12 Stokes, D.M. and Walker, D.A. (1971) Plant Physiol. 48, 163–165
13 Takahama, U., Shimizu-Takahama, M. and Heber, U. (1981) Biochim. Biophys. Acta 637, 530–539
14 Clayton, R.K. (1980) Photosynthesis. Physical Mechanisms and Chemical Patterns, Cambridge University Press, Cambridge
15 Witt, H.T. (1971) Q. Rev. Biophys. 4, 365–477
16 Böhme, H. (1978) Prep. J. Biochem. 88, 137–141
17 Sivak, M.N. and Walker, D.A. (1985) Plant, Cell Environ. 8, 439–448
18 Franck (1953) Arch. Biochem. Biophys. 45, 190–229
19 Heber, U. and Santarius, K.A. (1966) in Currents in Photosynthesis, pp. 993–400, A.D. Donker, Rotterdam.
20 Stitt, H., Wirtz, W. and Heldt, H.W. (1980) Biochim. Biophys. Acta. 593, 85–102
21 Badger, M.R., Sharkey, T.D. and Von Caemmerer, S. (1984) Planta 160, 305–313
22 Stitt, M., Kürzel, B. and Heldt, H.W. (1984) Plant Physiol. 75, 554–560
23 Lilley, R.McC., Chon, C.J., Mosbach, A. and Heldt, H.W. (1977) Biochim. Biophys. Acta 460, 259–72
24 Santarius, K.A. and Heber, U. (1965) Biochim. Biophys. Acta. 102, 39–54
25 Baldry, C.W., Walker, D.A. and Bucke, C. (1966) Biochem. J. 101, 642–646
26 Walker, D.A., Kosciukiewicz, K. and Case, C. (1973) New Phytol. 72, 237–247
27 Walker, D.A. (1976) in The Intact Chloroplast (Barber, J. ed.), pp. 235–278, Elsevier, Amsterdam
28 Delieu, T.J. and Walker, D.A. (1983) Plant Physiol. 73, 534–541
29 Stitt, M., Wirtz, W., Gerhardt, R., Heldt, H.W., Spencer, C., Walker, D.A. and Foyer, C.H. (1985) Planta 166, 354–364
30 Foyer, C.H. and Spencer, C. (1986) Planta, in the press
31 Dietz, K.-J. and Foyer, C.H. (1986) Planta in the press
32 Sharkey, T.D. (1985) Plant Physiol. 78, 71–75
33 Robinson, S.P. and Wiskich, J.T. (1976) Biochim. Biophys. Acta 440, 131–146
34 Robinson, S.P. and Walker, D.A. (1979) Biochim. Biophys. Acta 545, 528–536.
35 Carver, K.A., Hope, A.B. and Walker, D.A. (1983) Biochem. J. 210, 273–276
36 Heber, U., Takahama, U., Neimanis, S. and Shimizu-Takahama, M. (1982) Biochim. Biophys. Acta 679, 287–299