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Studies on the induction of chlorophyll fluorescence in barley protoplasts. III. Correlation between changes in the level of glycerate 3-phosphate and the pattern of fluorescence quenching

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Metabolite levels were measured in dark-adapted, isolated barley protoplasts exposed to illumination. The level of glycerate 3-phosphate showed a large peak 2 min after the onset of illumination in protoplast preparations which displayed an oscillatory transient in the rate of O_2 evolution and in the yield of chlorophyll fluorescence (Quick, W.P. and Horton, P. (1984) Proc. R. Soc. Lond. B. 220, 361–370). In protoplasts which did not show this oscillatory behaviour the level of glycerate-3-P steadily rose to its steady-state value. The relationships between electron transfer and carbon assimilation that underlie these transients in O_2 evolution, chlorophyll fluorescence and glycerate-3-phosphate level are discussed.

Introduction

Upon illumination of isolated protoplasts following a prolonged dark period, the acceleration in the rate of O_2 evolution has been observed to be interrupted by a transient, creating a pattern that resembles a heavily damped oscillation [1–3]. The accompanying quenching of chlorophyll fluorescence is also oscillatory [2], and analysis has shown that changes in both the redox state quenching (q_Q) and the ΔpH-induced quenching (q_e) contribute to the transient [3]. Similar phenomena have been characterised in leaves, with assays of light scattering [4] supplementing previous simultaneous measurements of chlorophyll fluorescence, O_2 evolution and CO_2 uptake [5,6]. It has been proposed that these oscillations reside in the control of flux through the reductive pentose phosphate pathway and are induced when imbalance is generated between ATP consumption by glycerate-3-P kinase and by Rbu-5-P kinase [5–8]. Even though oscillations may not occur under most physiological conditions, these phenomena express important information on the regulation and limitation of photosynthesis; for example, it has become clear that regulatory mechanisms restrict the expression of the maximum capacities for electron transfer and carbon assimilation [9]. This model for oscillations, which may also include interactions with control over the relative proportions of linear and cyclic electron flow [10], suggests that the levels of reductive pentose phosphate pathway metabolites, especially glycerate-3-P, would fluctuate during the oscillation. In this paper, it is demonstrated that the level of glycerate-3-P shows a pronounced peak which is correlated with the

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Abbreviations: Fru-1,6-P_2, fructose-1,6-bisphosphate; glycerate-3-P, glycerate 3-phosphate; Rbu-5-P, ribulose-5-phosphate; Rbu-1,5-P_2, ribulose 1,5-bisphosphate; triose-P, triose phosphate; Chl, chlorophyll.

occurrence of the oscillatory transient in fluorescence quenching and the rate of O_2 evolution.

Materials and Methods

Protoplasts were isolated from barley as described previously [2] and fluorescence and O_2 evolution assayed in a modified Hansatech O_2 electrode [2,3] at 20°C and under 120 $W \cdot m^{-2}$ of blue light. Samples for metabolite assays were prepared by transferring, under vacuum, 1 ml of protoplast suspension (100 μg Chl/ml) from the electrode chamber into perchloric acid. Transfer times were less than 1 s and the time spent in the transit tube, which was also illuminated at the same intensity as the sample chamber, was calculated to be less than 100 ms. After neutralisation with KOH, assays were carried out enzymically [11] by following NADH absorption in a Bristol dual-wavelength spectrophotometer (340 nm minus 400 nm). ATP and ADP were measured by the luciferase technique [12].

Results

Barley protoplasts prepared from plants grown either at different times of the year in the greenhouse or under artificial lighting in a growth chamber exhibit varying degrees of oscillatory behaviour. Although the reasons for such differences have not been established, they have been used to aid the investigation of the relationship between metabolite levels and the behaviour of O_2 evolution and fluorescence quenching during induction. Fig. 1a shows the previously reported pattern of fluorescence quenching and the rate of O_2 evolution observed upon illumination of dark-adapted protoplasts [2]. A peak in the rate of O_2 evolution and a fluorescence minimum were observed after approx. 2 min, whereas a trough in O_2 evolution and a fluorescence maximum occurred after 2.5–3.0 min. Note the previously reported phase shift between the O_2 and fluorescence signals [2,3].

The level of ATP was found to increase to a maximum within the first minute of illumination and subsequently to decline between 1 and 2 min as the rate of O_2 evolution accelerated. The ADP level was approximately complementary to that of ATP and, although a shallow rise in ATP was observed beyond 3 min, no clear oscillatory behaviour was detected (Fig. 1b). The levels of glycerate-3-P and Rbu-1,5-P_2 during induction are shown in Fig. 1c. The level of Rbu-1,5-P_2 doubled during the induction period and rose gradually to the steady-state value. There was some decrease in the level between 1 and 3 min, the period of the oscillatory transient seen in Fig. 1a, but the changes involved are small and approach the resolution of the assay technique. In contrast, the concentration of glycerate-3-P showed a well-defined transient; during the first 2.5 min, it rose from a low dark level to a value more than double that found in the final steady state. The fall to the final steady-state level was rapid between 2 and 3 min and then slower between 3 and 7 min. The peak in the level of glycerate-3-P (2.25 min) coincided with the time of the oscillatory changes in rate of O_2 evolution and fluorescence shown in Fig. 1a. The glycerate-3-P peak corresponded to the top of the fluorescence transient (called M_2 and occurring at 2.25 min) and the minimum in the rate of O_2 evolution (2.5 min). The fluorescence minimum (S_2) was associated with the maximum rate of increase in the level of glycerate-3-P, and as observed previously, a maximum rate of O_2 evolution [2]. The decline in glycerate-3-P was then accompanied by further quenching from M_2 to T, the steady-state fluorescence level, and the associated acceleration in the rate of O_2 evolution to its steady-state rate.

Fig. 1d shows the changes in Fru-1,6-P_2 and triose-P during the induction period. The level of Fru-1,6-P_2 doubled within 2 min of illumination and thereafter remained constant. Triose-P exhibited a rise from an initial low level to a value some 10-times larger after 6 min illumination. After an initially rapid rise, the rate of triose-P accumulation slowed between 1 and 3 min before resuming at a faster rate after 3 min; this decrease in rate of triose-P accumulation occurred during the period of the oscillation when the rate of O_2 evolution decreased.

Fig. 2 shows the results of similar assays carried out on protoplasts that exhibited an induction period which did not show the oscillatory transient seen in Fig. 1. The oscillatory transient was not observed even if the illumination was extended to 20 min. Thus, after the initial characteristic burst

in O_2 evolution [2], the rate of O_2 evolution rose smoothly to its steady-state level without the presence of the oscillation seen in Fig. 1a (Fig. 2a). Similarly, the fluorescence was seen to be quenched mono-phasically, antiparallel to the increase in rate of O_2 evolution and without exhibiting the secondary oscillatory transient. The level of glycerate-3-P, although rising sharply as the rate of O_2 evolution accelerated, did not show the subsequent decline that was observed in Fig. 1c (Fig. 2b). For comparison the levels of Fru-1,6-P_2 and triose-P are shown (Fig. 2b); the changes in Fru-1,6-P_2 were largely similar to those shown in Fig. 1d, except that a fall in level was routinely seen between 3 and 5 min. Triose-P appeared to accumulate at a slow, but steady rate between 2 and 6 min and its accumulation did not show the same changes in rate observed in Fig. 1d.

It seems, therefore, that the transient rise and fall in level of glycerate-3-P is associated with the

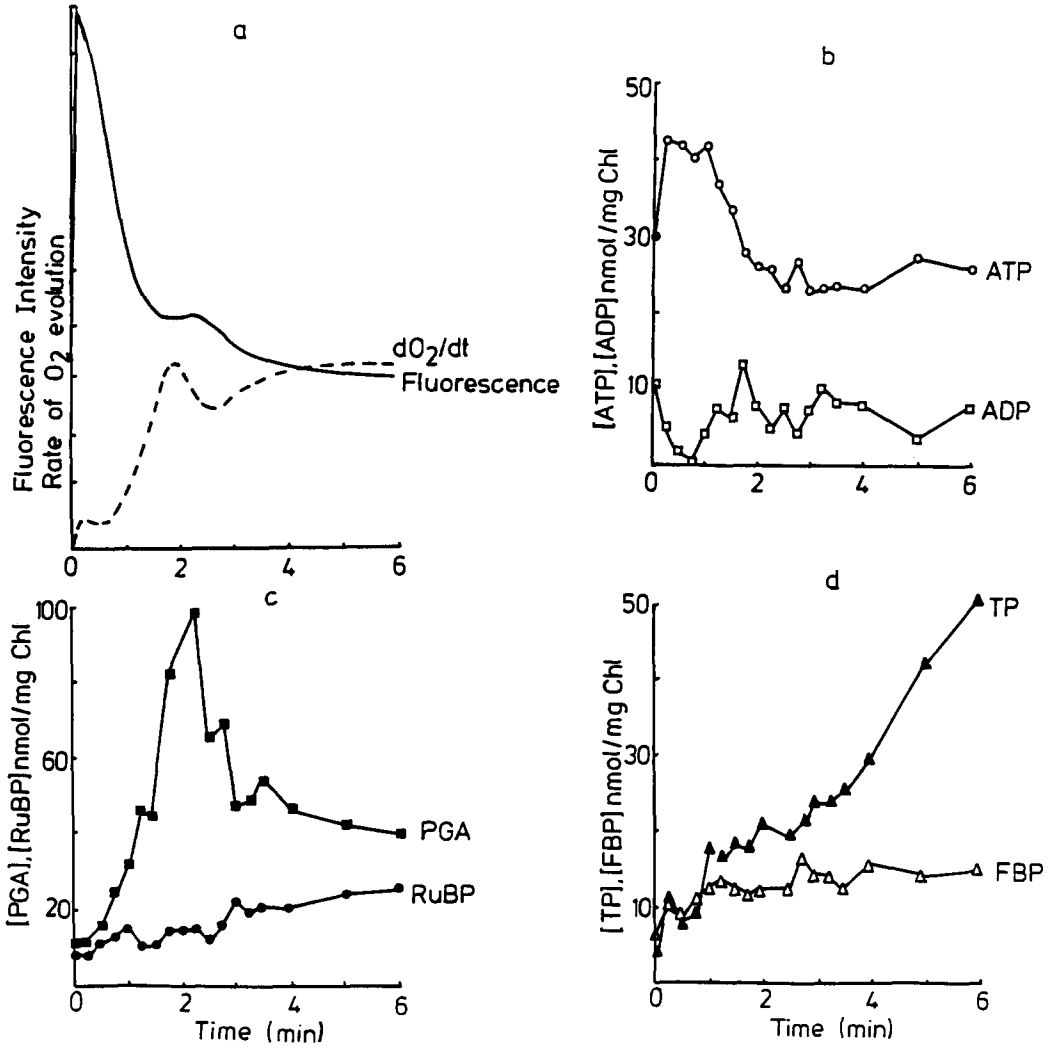

Fig. 1. Measurements of (a) the rate of O_2 evolution (dO_2/dt) and chlorophyll fluorescence, (b) levels of ATP and ADP, (c) levels of glycerate-3-P (PGA) and Rbu-1,5-P_2 (RuBP) and (d) levels of triose-P (TP) and Fru-1,6-P_2 (FBP) in barley protoplasts illuminated at time 0. In (a) fluorescence is given in relative units and the maximum steady-state rate of O_2 evolution was approx. 120 μmol O_2 per mg Chl per h. For further details refer to the text.

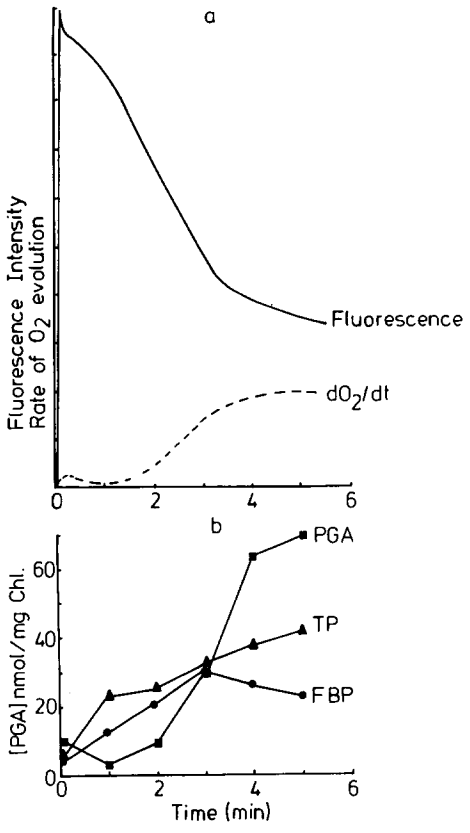

Fig. 2. Measurements of (a) the rate of O_2 evolution and chlorophyll fluorescence and (b) the levels of glycerate-3-P (PGA), triose-P (TP) and Fru-1,6-P_2 (FBP) in barley protoplasts illuminated at time 0. For further details, see Fig. 1 and the text.

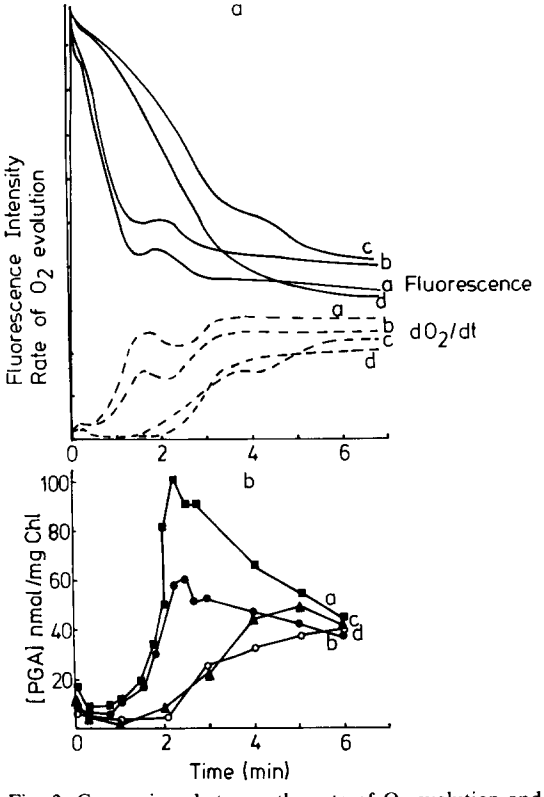

Fig. 3. Comparison between the rate of O_2 evolution and the yield of chlorophyll fluorescence (a) and the level of glycerate-3-P (PGA) (b) during induction in different preparations of protoplasts. For details refer to Fig. 1 and the text.

oscillatory behaviour in the rate of O_2 evolution and the extent of fluorescence quenching. In Fig. 3, the correlation has been strengthened by the results from four different protoplast preparations, each showing differences in the extents and timing of oscillation. The relative difference between the maximum and steady-state levels of glycerate-3-P as well as the time of occurrence of the peak correlated well with the extent and timing of the oscillatory transient in fluorescence intensity and rate of O_2 evolution.

Discussion

In an earlier study using protoplasts, it has been shown that the quenching of chlorophyll fluorescence accompanied the acceleration of O_2 evolution [2,3]. This link between fluorescence and carbon assimilation was implicit in earlier work on leaves [13] and has subsequently been re-stated in a range of studies on isolated chloroplasts [14–17], protoplasts [2,3] and leaves [4–9]. This relationship exists because the fluorescence yield is determined for the most part by the redox state of Q and the size of ΔpH, both of which are influenced by the rates of consumption of NADPH and ATP, respectively, during CO_2 assimilation (for a review, see Ref. 10).

In this study, it has been shown that the increase in the level of glycerate-3-P that occurs upon illumination of dark-adapted protoplasts can show the complex kinetics that accompany the oscillatory transient in both the rate of O_2 evolution and the extent of fluorescence quenching. Recent experiments using leaves have also shown large changes in the level of glycerate-3-P accompanying the oscillation in fluorescence induced by

a transition from air to 5% CO_2 [18]. The extent of the transient in the level of glycerate-3-P and the fluctuations in fluorescence and the rate of O_2 evolution were strongly correlated. Thus, when oscillatory behaviour was undetectable, the level of glycerate-3-P rose smoothly to its steady-state value. In previous work, glycerate-3-P has been shown to rise monophasically during induction [19,20] in systems where the rate of O_2 evolution also accelerated without oscillation. It cannot be ascertained whether the changes in glycerate-3-P levels shown here occurred in the chloroplast or cytoplasm. Although light-induced alkalinisation of the stroma restricts export [21], increases in the level of glycerate-3-P in both the cytoplasmic and chloroplastic compartments have been observed during induction [19,20]. Similar problems arise when considering some of the other metabolites. The level of triose-P increased with complex kinetics in Fig. 1. These kinetics could be explained by the observation that chloroplastic triose-P increased rapidly and the cytoplasmic pool more slowly upon illumination [19]. However, the rate of accumulation of triose-P closely follows the rate of O_2 evolution during the oscillation, suggesting that triose-P synthesis in the chloroplast is the cause of this complex behaviour. Supporting this conclusion are the much simpler kinetics of triose-P accumulation in protoplasts not exhibiting oscillatory induction. Rbu-1,5-P_2 is located exclusively in the chloroplast [20] and the changes seen in the levels of Rbu-1,5-P_2 shown in Fig. 1 are similar to those reported previously [19,20].

The rapid rise in ATP followed by a gradual fall as the rate of O_2 evolution accelerates is a phenomenon routinely observed in chloroplasts, protoplasts and leaves [22]. The fall in ATP, which is reflected in chloroplasts by the size of the transthylakoid ΔpH as measured by 9-aminoacridine quenching [15,23,24] is considered to reflect increased consumption as the rate of carbon assimilation accelerates. Indeed, the decline in ATP shown in Fig. 1 coincided with a period of rapid acceleration in the rate of O_2 evolution. It is perhaps surprising that there was no detectable transient in the level of ATP when the rate of photosynthesis was seen to oscillate. Previous work has shown that changes in the level of ATP were faster and larger in the chloroplast compartment than in the cytoplasm [19], but it is possible that a high ATP/ADP ratio in the cytoplasm could mask small transients in the chloroplast adenylate pools.

The mechanism which underlies the relationship between the changes in chlorophyll fluorescence, the rate of photosynthesis and the level of glycerate-3-P should now be discussed. The level of glycerate-3-P is obviously a balance between its production by carboxylation of Rbu-1,5-P_2 and its conversion to triose-P. In the absence of a significant export of carbon from the chloroplast, glycerate-3-P levels will rise rapidly as photosynthesis accelerates. A continued increase in glycerate-3-P in the presence of a deceleration in O_2 evolution and a decreased rate of triose-P accumulation, as occurred during the oscillation, indicates that the reduction of glycerate-3-P has been inhibited by a deficiency in ATP or NADPH. An alternative view that restricted electron flow results from a limitation of Rbu-1,5-P_2 regeneration or carboxylation is clearly inconsistent with the observed changes in glycerate-3-P. In due course, glycerate-3-P production would also be impaired as regeneration of Rbu-1,5-P_2 decreased and subsequently, the level of glycerate-3-P would fall. The peak in level observed during the oscillation is therefore consistent with the hypothesis put forward that the increased consumption of ATP by Rbu-5-P kinase (caused as a result of an enhanced rate of glycerate-3-P production and conversion to Rbu-5-P) results in the ATP/ADP ratio decreasing sufficiently to impede glycerate-3-P reduction [5–9]. However, the absence of significant transitions in the level of ATP would tend to argue against this proposal, although it should be added that the q_e (ΔpH-induced quenching) component of the fluorescence signal has been shown to oscillate; a minimum ΔpH was associated with the minimum in the rate of O_2 evolution, preceding it by 10–15 s [3]. Moreover, it is unlikely that inhibition of glycerate-3-P reduction could result from a deficiency in NADPH, especially when ΔpH is sub-maximal. However, from the available data, cause and effect cannot be easily distinguished and it is therefore impossible to assess the relative contributions to oscillatory behaviour from electron transfer, the reductive pentose phosphate pathway and enzyme activation steps distant from both (e.g., sucrose synthesis).

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