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An analysis of the chlorophyll-fluorescence transients from pea leaves generated by changes in atmospheric concentrations of CO_2 and O_2

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Removal of CO_2 from pea leaves, which were either grown in a glasshouse at 15°C or in a controlled environment at 23°C, produced an initial increase in chlorophyll fluorescence emission followed by a slow decrease to steady state. From estimations of the redox state of Q, using a nondestructive in vivo technique, the contributions of photochemical and nonphotochemical quenching processes to these fluorescence transients were determined. The fluorescence changes observed on removal of CO_2 from the two types of pea leaves were mainly attributable to changes in nonphotochemical quenching although markedly different changes in photochemical quenching were also observed. When leaves grown at 23°C were depleted of CO_2, Q immediately became more reduced, whereas in leaves grown at 15°C Q, unexpectedly, became more oxidised. On return of CO_2 to the leaves these phenomena were reversed, i.e., in leaves grown at 23°C Q became more oxidised and in the 15°C grown leaves Q became more reduced. Increased electron transport to O_2 may account for the oxidation of Q on depletion of CO_2 from 15°C grown leaves. The generation of fluorescence transients on removal and return of CO_2 to the leaf required the presence of oxygen. The fast fluorescence kinetics observed on exposure of the leaf at steady state to a second saturating irradiation suggest that O_2 may accept electrons directly from Photosystem II at a site between Q and B.

Introduction

Recent observations that changes in chlorophyll-fluorescence emission from photosynthetic systems are closely correlated with changes in the rates of O_2 evolution [1–6] and CO_2 assimilation [1,7,8] have led to speculations that fluorescence transients contain information on the regulatory mechanisms involved in photosynthesis. Under physiological conditions the level of chlorophyll fluorescence emission can be modified by both photochemical and nonphotochemical processes. Photochemical quenching, termed q(P) and often referred to as q_Q (e.g., Refs. 3 and 9), results from oxidation of Q, whilst nonphotochemical quenching, q(nP), can be produced by generation of a transthylakoid membrane ΔpH, excitation energy transfer from PS II pigment matrices to pigment beds having a lower quantum yield of fluorescence, photoinhibition of PS II and oxidised plastoquinone. Generally, the major nonphotochemical quenching mechanism in physiologically active photosynthetic systems is considered to be the transthylakoid pH gradient [4,9]. This type of non-photochemical quenching has been termed q_e (e.g., Refs. 3 and 9); however, it should be emphasised that although q_e may constitute a major proportion of q(nP) in many situations it should not be equated to q(nP), since q(nP) can be mod-

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Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; q(P), photochemical quenching; q(nP), nonphotochemical quenching.

ified by other quenching mechanisms. Changes in q(P) are related to changes in the rate of noncyclic electron transport and the associated proton pumping across the thylakoids [9]. However, the magnitude of the transthylakoid membrane ΔpH will also depend upon the rate of dissipation of the proton electrochemical gradient by phosphorylation of ADP, which in turn will depend on the phosphorylation potential (ATP/ADP + P_i) of the stroma. Although changes in q(nP) will be related to those in q(P), the relationship will be complex and determined by many factors.

Particularly interesting fluorescence transients, with respect to the relationship between q(P) and q(nP), are generated when the CO_2 concentration in the atmosphere surrounding leaf tissue is rapidly changed. When spinach leaves at a steady-state fluorescence level in air are deprived of CO_2, a rapid increase in fluorescence is observed followed by a slow decline to a lower steady-state level [12]. The rapid rise has been explained by a fast decrease in q(P) which occurs as a result of an increase in NADPH due to a decrease in the amount of 3-phosphoglycerate available for reduction. The slow decline in fluorescence on CO_2 removal has been attributed to an increase in q(nP) resulting from an increase in transthylakoid ΔpH, due to both an elevated rate of cyclic or pseudocyclic electron transport and a decrease in ATP consumption by carbon metabolism [12]. Measurements of the absorbance changes at 535 nm induced in spinach leaves by CO_2 depletion, which are considered to be indicative of changes in thylakoid energisation due to changes in the transthylakoid proton gradient and the adenylate status of the stroma [13,14], provide support for this hypothesis [15]. Returning CO_2 to the atmosphere surrounding the leaf produces a reversal of the transient observed on removing CO_2 although the magnitude of the transient is considerably less [12]. This transient has been explained in terms of increasing NADPH and ATP consumption which increase q(P) and decrease q(nP), respectively [12]. The differences in the magnitude of the transients observed on depleting and returning CO_2 to the leaf have been taken to suggest that q(nP) takes longer to manifest itself in the absence of CO_2 than it does to relax in the presence of CO_2. Thus, any switch from noncyclic to cyclic electron transport is less rapid than the increased consumption of ATP that occurs when CO_2 is returned [12]. As yet this hypothesis, with its important implications to photosynthetic control, has not been experimentally tested due to the absence of a satisfactory technique for determining q(P) and q(nP) for leaf tissue. In a previous paper we reported a non-destructive method for estimating q(P) and q(nP) during fluorescence transients from leaf tissue [11]. In this paper we use this method to estimate the changes in q(P) and q(nP) which occur during the fluorescence transients generated when the atmosphere of a leaf is depleted of CO_2 and then the CO_2 returned.

The data presented demonstrate that, although the above hypothesis does explain the nature of the fluorescence transients generated on CO_2 removal from pea leaves grown at 23°C, this does not account for similar fluorescence transients produced from pea leaves grown at 15°C. The role of O_2 in determining the nature of such fluorescence transients is also considered.

Materials and Methods

Plants of *Pisum sativum* cv. meteor were grown from seed in John Innes number 2 potting compost. Plants were grown either in a glasshouse under natural daylight during early spring at a mean temperature of 15°C or in a controlled growth cabinet under a 16 h photoperiod at 23°C. Fully expanded leaves were excised at the petiole base from 14-day-old plants and hermetically sealed in a gas-exchange chamber (ADC Ltd.). The cut end of the petiole was held outside the gas-exchange compartment and immersed in circulating deionised water to maintain leaf turgor. The temperature of the chamber was maintained at 23°C by circulating a heating fluid around the aluminium base of the chamber.

The flow rates of carbon dioxide, oxygen and nitrogen were monitored and accurately controlled by thermal mass flow controllers (Tylan Inc.) operated in conjunction with solenoid valves. The gases were mixed at known flow rates to produce specified gas compositions. The gas flow passed across the leaf in the chamber and through an infrared gas analyser (ADC Ltd.) operating in absolute mode to measure accurately the CO_2 con-

centration in the gas flow. Gas flow rate was 5 $cm^3 \cdot s^{-1}$ and unless stated otherwise CO_2 was maintained at 640 $mg \cdot m^{-3}$ (approximate atmospheric concentration) and O_2 at 20%. Infrared gas analyzer measurements indicated that the CO_2 level could be reduced from 640 to less than 10 $mg \cdot m^{-3}$ in 5 s, and could be brought back to ambient levels within 30 s.

Broad-band blue light from two quartz-iodine sources (Volpi) was passed through a heat reflecting mirror (Ealing Beck) and sharp 580 nm cut-off filter (Ealing Beck) mixed and passed to the leaf chamber by a randomised bifurcated light guide. The emitted fluorescence was transferred by a second fibre optic to a photomultiplier (R446, Hakuto International) protected by a 680 nm interference filter (Ealing Beck). The signal from the photomultiplier was amplified and transferred to a digital storage oscilloscope to measure fast fluorescence transients. The continuous fluorescence signal was recorded on a potentiometric chart recorder.

Photochemical quenching was estimated in vivo by a technique described previously [10,11] in which a continuously illuminated leaf was exposed to a second saturating excitation. On addition of this second irradiation, fluorescence rises rapidly to a level designated as F_{O2} and increases further to a maximum level at F_{P2}, thus generating an additional variable fluorescence, F_{V2}, where $F_{V2} = F_{P2} - F_{O2}$. Because the additional excitation maximally reduces the PS II electron acceptor pool, the redox state of Q at the point of addition is estimated by F_{V2}/F_{O2}. A value for F_{V2}/F_{O2} of zero indicates complete reduction of Q and increasing values of F_{V2}/F_{O2} indicate increasing amounts of oxidised Q. The level of fluorescence that would occur if PS II traps were maximally closed, $F(Q_{red})$, can then be obtained from the additional variable fluorescence and the level of fluorescence at the point of addition of the second irradiation, F(add), [10,11]. Photochemical quenching, q(P), is then given by:

$$q(P) = F(Q_{red}) - F(add) \quad (1)$$

and nonphotochemical quenching, q(nP), is estimated by:

$$q(nP) = q(tot) - q(P) \quad (2)$$

where q(tot) is the total amount of fluorescence quenching. Estimation of q(P) by this method is dependent upon F_{V2}/F_{O2} relating directly to the redox state of Q. Values of $F(Q_{red})$ calculated from F_{V2}/F_{O2} during fluorescence quenching in isolated chloroplasts were similar to the fluorescence levels rapidly attained on reduction of Q by addition of DCMU [16]. Also, determinations of the fluorescence quenching due to oxidised Q during the fluorescence induction curve of barley protoplasts by a light addition technique correlated strongly with measurements made using DCMU [3].

The initial continuous illumination was provided at 100 μmol photons per m^2 per s and the second excitation provided at 500 μmol photons per m^2 per s. The F_{O2} and F_{P2} levels of fluorescence were measured using a digital storage oscilloscope triggered by an electronic shutter (Ealing Beck) controlling the second excitation. Values for F_{V2}/F_{O2} and the estimations of q(P) and q(nP) were thereby obtained during the various transients generated by removal or addition of CO_2 and O_2.

Results

Removal of CO_2 from the atmosphere of a pea leaf, grown at 23°C, produced an increase followed by a decrease in fluorescence emission (Fig. 1a) and the steady-state fluorescence level of the pea leaves in the absence of CO_2 was found to be slightly lower than that prior to the removal of CO_2, as was found previously to be the case for spinach leaves [12]. However, the kinetics of the transients from pea leaves (Fig. 1a) are rather slower than those reported for spinach leaves [12]. Such kinetic differences may be attributable to metabolic and anatomical differences between the two species and/or a slower rate of removal of CO_2 from the atmosphere of the pea leaf. By exposing the pea leaves to a second excitation, which was saturating for the ratio of variable, F_{V2}, to nonvariable, F_{O2}, fluorescence, the redox state of the PS II electron acceptors at any given time could be estimated as explained in the Materials and Methods section. The changes in F_{V2}/F_{O2}, which relate to changes in the redox state of Q, throughout the fluorescence changes generated on

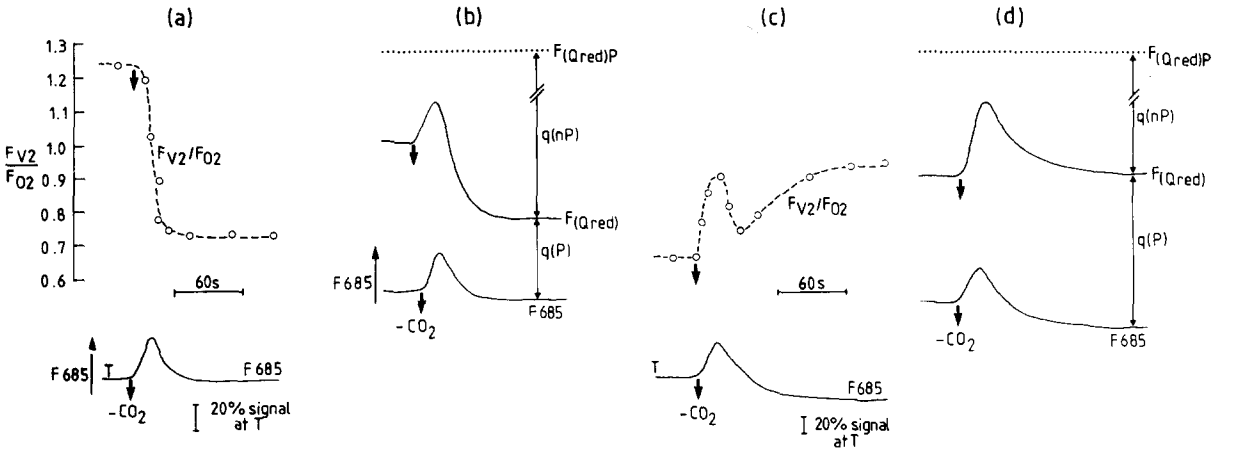

Fig. 1. Changes in fluorescence (F_{685}) and F_{V2}/F_{O2}, which estimates the redox state of Q, induced by the removal of CO_2 from pea leaves, grown in a controlled environment cabinet at 23°C (a) and in a glasshouse at 15°C (c), when the leaves had reached a steady-state fluorescence level at T. The fluorescence level that would occur if Q were maximally reduced, $F(Q_{red})$, was determined as outlined in the Materials and Methods section and is shown together with the changes in photochemical, q(P), and nonphotochemical, q(nP), quenching during the fluorescence transients induced on removal of CO_2 from 23°C (b) and 15°C (d) grown pea leaves. $F(Q_{red})$P, which designates the level of fluorescence attained at the point P on the induction curve if Q was maximally reduced, is a reference point for the calculation of q(P) and q(nP) (see Refs. 10 and 11). The vertical bar represents 20% of the fluorescence level measured at the point T on the induction curve.

depletion of CO_2 are shown in Fig. 1a.

From a knowledge of the fluorescence emission level at the point of addition of the second irradiation and the value of F_{V2}/F_{O2} produced by the second excitation, the fluorescence level which would be attained if Q was maximally reduced, i.e. $F(Q_{red})$, can be calculated [10,11]. The difference between $F(Q_{red})$ and the observed fluorescence level prior to the addition of the second excitation then estimates the amount of fluorescence that has been quenched by photochemical processes, q(P) [11]. Nonphotochemical quenching, q(nP), is then estimated by the difference between the total amount of quenching observed and q(P); see the Materials and Methods section. The calculated change in q(P) and q(nP) throughout the fluorescence transients generated on depletion of CO_2 from the leaf grown at 23°C are shown in Fig. 1b.

Although a decline in F_{V2}/F_{O2} is observed immediately on removal of the CO_2 (Fig. 1a), indicating a partial reduction of Q, this produces only a negligible change in q(P), as shown by the negligible change in the difference between the levels of F_{685} and $F(Q_{red})$. Simultaneously, however, there is a large decrease in q(nP), which is clearly the major contributor to the overall fluorescence increase. The decline in the fluorescence level after reaching a maximum is accompanied by a decrease in q(P) and a considerably greater increase in q(nP). The decrease in q(P) can be attributed almost totally to the depression of the fluorescence level of the leaf by the increased q(nP), since F_{V2}/F_{O2} does not change markedly during this period; q(P) is a function of both F_{V2}/F_{O2} and the fluorescence level of the leaf at the point of addition of the second excitation [11]. This data suggests that although changes in q(P) do occur during the fluorescence transients generated on removal of CO_2 from the leaf, it is the considerably greater changes in q(nP) that determine the nature of the transients.

When these experiments were repeated on pea leaves grown at 15°C in a glasshouse, rather than at 23°C in a controlled environment cabinet, similar fluorescence transients were observed to those reported above, however, the changes in F_{V2}/F_{O2}, q(P) and q(nP) throughout these transients were remarkably different (Fig. 1c and d). Immediately on removal of the CO_2 from the 15°C grown leaf an increase in F_{V2}/F_{O2} was observed, indicating surprisingly that an oxidation, rather than a reduction, of Q was occurring. This produced an increase in q(P); however, the simultaneous decrease in q(nP) was considerably greater resulting in a net

increase in the observed fluorescence level. This is a particularly interesting situation, since it demonstrates that situations can arise in which photochemical quenching increases but a simultaneous and greater decrease in nonphotochemical quenching can overcome this effect and lead to an overall net increase in the observed fluorescence level. After the leaf fluorescence level reaches a maximum, there is a slow decline until a steady state is reached. During this decline there is a reduction followed by a further oxidation of Q, as shown by the oscillations in F_{V2}/F_{O2}. However, such changes in the redox state of Q make only a small contribution to the change in the fluorescence level, i.e., only small changes in q(P) are observed during this phase. The major factor contributing to the fluorescence decrease is the large increase in q(nP).

On returning CO_2 to a pea leaf grown at 23°C and previously depleted of CO_2 an increase in fluorescence was observed during which F_{V2}/F_{O2} increased and then decreased to a lower level than that previously observed in the absence of CO_2 (Fig. 2a). F_{V2}/F_{O2} then increased in conjunction with a small decline in the level of fluorescence. However, the F_{V2}/F_{O2} value of approx. 0.77 at steady state, after CO_2 was reintroduced to the leaf (Fig. 2a), was considerably lower than the F_{V2}/F_{O2} value of approx. 1.24, found before CO_2 was removed (Fig. 1a), implying that Q was considerably more reduced at steady state after reintroduction of CO_2 than prior to CO_2 depletion. In a 15°C grown pea leaf, previously depleted of CO_2, the value of F_{V2}/F_{O2} after reintroduction of CO_2 was found to be identical to that observed prior to CO_2 removal (Figs. 1c and 2c). During the fluorescence rise produced by reintroduction of CO_2 to a 23°C grown leaf, q(P) increased and q(nP) declined (Fig. 2b). On readmission of CO_2 to a 15°C grown leaf, changes in q(P) and q(nP) were found to be opposite to those observed in the 23°C grown leaf; that is q(P) decreased, while q(nP) increased. The large increase in q(nP) observed on CO_2 readmission to a 15°C grown leaf produces such a large decrease in the fluorescence level that $F(Q_{red})$ actually decreases during this phase despite the reduction of PS II electron acceptors as shown by the decrease in F_{V2}/F_{O2} (Fig. 2c and d). The fluorescence rise that follows the decrease on reexposure of a 15°C grown leaf to CO_2 is clearly attributable to a decrease in q(nP), since q(P) remains constant during this phase (Fig. 2d). These transients which result from readmission of CO_2 to the leaf do not appear to be simply the converse of the fluorescence transients found on removal of CO_2 as previously suggested [12].

The increase in F_{V2}/F_{O2} observed immediately on removal of CO_2 from the 15°C grown leaf (Fig. 1c) argues for an increase in electron flow away from Q on CO_2 depletion, which presumably is the result of an increase in electron transport to another terminal electron acceptor, possibly O_2. The reverse of this phenomenon is observed when CO_2 is returned to the leaf and F_{V2}/F_{O2} decreases (Fig. 2c), implicating a reduction rather than the expected oxidation of Q as CO_2 removes electrons. These data strongly suggest that electron transport

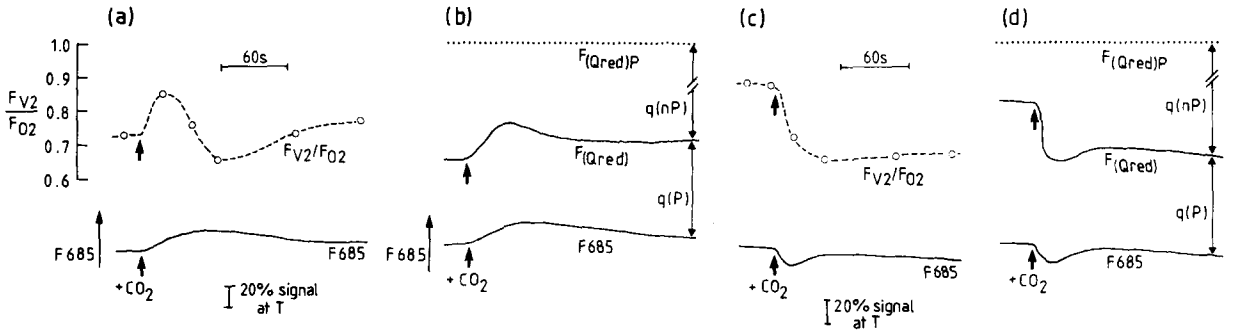

Fig. 2. Changes in fluorescence parameters induced by the reintroduction of CO_2 to CO_2-depleted pea leaves grown at 23 (a,b) and 15°C (c,d). Changes in fluorescence (F_{685}) and F_{V2}/F_{O2} for leaves grown at 23 and 15°C are shown in (a) and (c), respectively. Changes in q(P) and q(nP) for leaves grown at 23 and 15°C are shown in (b) and (d), respectively. Refer to Fig. 1 for other details.

to a terminal electron acceptor, that is not CO_2, can be sustained in vivo at rates sufficiently large to maintain Q in a partially oxidised state.

It is well established that the rapid fluorescence induction transients (O,I,D,P) generated on exposure of dark-adapted intact photosynthetic systems contain information on the nature of the electron flow into and out of Q [17,19,20]. Similarly, it can be argued that the fluorescence transients generated on exposure of an irradiated leaf to a second, high intensity excitation must also contain information on the characteristics of electron flow into and out of Q. In the presence of CO_2 and at steady state fluorescence, the fluorescence transients generated from a 15°C grown leaf by the second irradiation (Fig. 3) are similar to those observed when a dark-adapted leaf is initially excited [21,22]. Immediately upon exposure to the second excitation, the fluorescence rises to the F_{O2} level, a slower rise to I_2 and a decline to D_2 then occur, followed by a considerably slower increase in fluorescence to the maximal, P_2, level. However, in the absence of CO_2 the induction curve generated by the second irradiation lacks the I_2 to D_2 transient and P_2 is reached in less than 2 s, considerably faster than observed

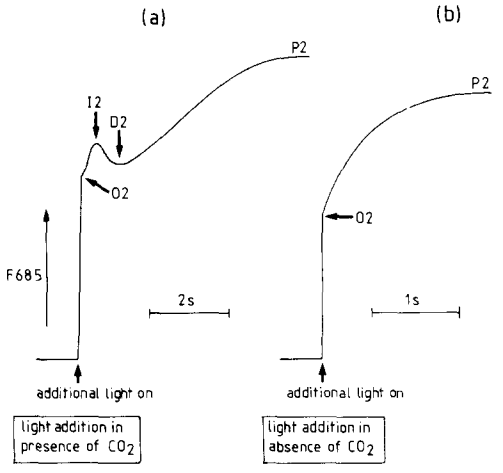

Fig. 3. Fluorescence transients produced by an additional excitation of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ given at the steady-state level of fluorescence from 15°C grown pea leaves in the presence (a) or absence (b) of CO_2. The fluorescence transients between O2, I2, D2 and P2 are clearly seen in the presence of CO_2 while only a monotonous rise from O2 to P2 occurs in the absence of CO_2. Note the different time scales in a and b.

in the presence of CO_2 (Fig. 3). At steady state in the presence of CO_2, F_{V2}/F_{O2} is approx. 0.70, whilst in the absence of CO_2 it is approx. 0.88, implying that Q is slightly more oxidised in the absence of CO_2 and thus would be expected to take longer to reduce maximally on exposure of the leaf to the additional excitation assuming similar electron-transport mechanisms in both cases; however, this is clearly not the case. It should be emphasised that an F_{V2}/F_{O2} value of 1.0 for leaf tissue implies that only about 30% of Q is oxidised [10], thus the difference in the proportion of Q oxidised in the presence and absence of CO_2 is only relatively small; clearly in both cases less than 30% of Q is oxidised.

Previous studies of the OID transient in intact chloroplasts and algae have attributed the I–D transient to oxidation of Q by an electron acceptor, presumably B, located between Q and PQ [17,19]. If B is maintained oxidised by pretreatment of the thylakoids with far-red light, then the I–D transient is diminished in size [17]. Conversely, reduction of B will enhance the I–D dip [17]. Pretreatment of dark-adapted 15°C grown pea leaves with either 10 s of broad band blue or far-red light (710 nM) to reduce or oxidise B, respectively, produced the predicted enhancement and reduction of the I–D transient (Fig. 4), suggesting that in the absence of CO_2 the loss of the I_2–D_2 transient may be due to an inability of Q to transfer electrons to B. This could be a consequence of either an inhibition of electron flow from Q to B or B being highly reduced prior to the second excitation. However, although B may be inefficient at removing electrons from Q in the absence of CO_2, clearly a mechanism for oxidising Q is in operation in the leaf under these conditions, since at the steady-state fluorescence level, prior to addition of the second excitation, Q is more oxidised in the absence, than in the presence of CO_2.

It has been suggested that O_2 may have an important role in the regulation of electron transport in vivo by acting as a terminal electron acceptor in a Mehler reaction [23,24] and/or as a substrate for ribulose 1,5-diphosphate oxygenase [25,28]. The possible involvement of O_2 in determining the nature of the fluorescence transients generated on the depletion of CO_2 from and its

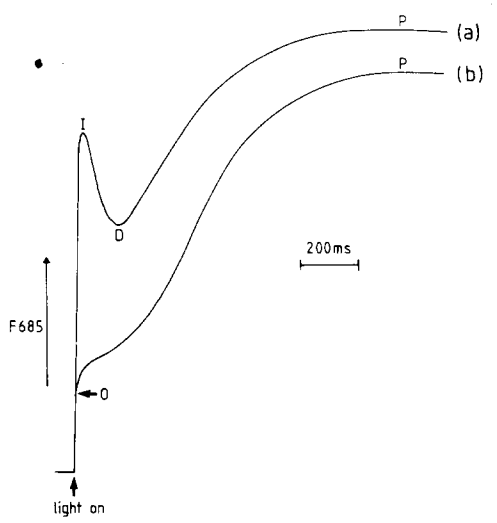

Fig. 4. The effect of pretreating a 15°C grown pea leaf with blue (less than 580 nm) (a) or far-red (710 nm) (b) radiation on the fluorescence induction kinetics induced by 100 μmol·m^{-2}·s^{-1} of blue radiation. Leaves were exposed to 10 s of broad-band blue (a) or far-red (b) radiation followed by a 5 s period of dark prior to exposure to blue radiation in order to record the 685 nm fluorescence induction curve.

return to the 15°C grown pea leaf was examined. The fluorescence-induction curve observed from a pea leaf in the presence and absence of O_2 is shown in Fig. 5. The rate of quenching from P in the absence of O_2 is considerably slower than observed in the presence of O_2, and the characteristic P, S, M, T transients are lost. Removal of CO_2 from the leaf produced no change in the rate of fluorescence quenching in the absence of O_2 (Fig. 5). Similarly, return of CO_2 to a CO_2-depleted leaf had no effect on the fluorescence signal. Addition of a second excitation to the leaf in the absence of O_2 produced negligible variable fluorescence; O_2-depleted leaves in the presence and absence of CO_2 exhibited F_{V2}/F_{O2} values of approx 0.05 (Fig. 5). These data demonstrate that Q is highly reduced in O_2-depleted leaves and CO_2 removal has no effect on the redox state of Q. However, removal of O_2 from a leaf depleted of CO_2 5 min prior to illumination results in a slow increase in fluorescence during which F_{V2}/F_{O2} decreases from

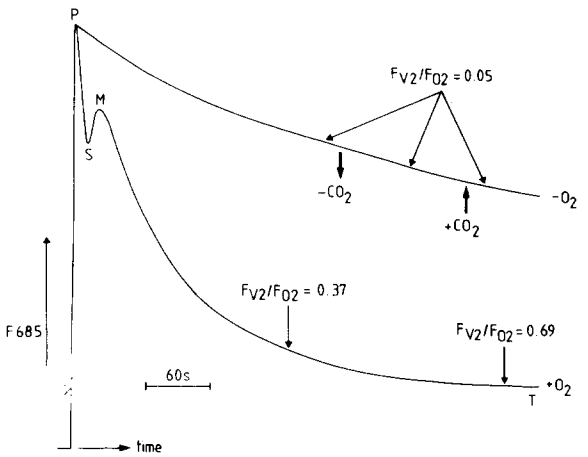

Fig. 5. The fluorescence induction curve from pea leaves grown at 15°C in the presence ($+O_2$) or absence ($-O_2$) of O_2. Removal and readdition of CO_2 to the anaerobic leaf had no effect on the rate of fluorescence quenching or on the magnitude of F_{V2}/F_{O2} produced by an additional excitation of 500 μmol·m^{-2}·s^{-1} (L2). Values of F_{V2}/F_{O2} at points during the fluorescence quenching are shown.

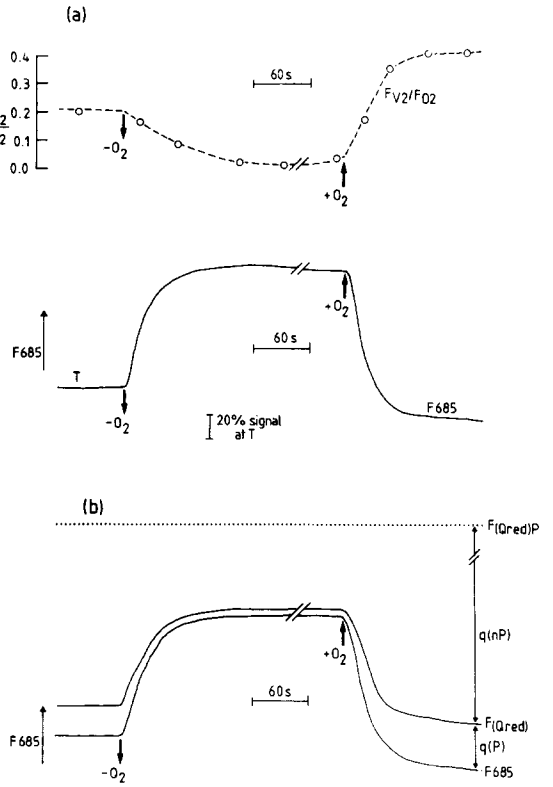

Fig. 6. Changes in fluorescence (F685), F_{V2}/F_{O2}, q(P) and q(nP) induced by the removal of O_2 from pea leaves (grown at 15°C) at steady-state photosynthesis and which had been depleted of CO_2 5 min prior to irradiation. See legend to Fig. 1 and the Materials and Methods section for definitions of $F(Q_{red})P$, $F(Q_{red})$, q(P) and q(nP).

approx. 0.21 to approx. 0.05, indicating a reduction of Q (Fig. 6). Calculations of q(P) and q(nP) throughout this transient demonstrate that both parameters decrease with time and contribute to the fluorescence increase although the change in q(nP) is greater than q(P) (Fig. 6). On the return of O_2 to the leaf a reversal of these effects is observed; both q(P) and q(nP) increase with q(nP) making the larger contribution to the fluorescence decline (Fig. 6).

Discussion

The nature of the fluorescence transients generated on removal and return of CO_2 to pea leaves are attributable to changes in both q(P) and q(nP) as predicted by Krause [15] and Sivak et al. [12]. However, it is evident that in pea leaves it is the changes in nonphotochemical quenching processes that make the major contribution to the changes in fluorescence emission. The relationship between q(P) and q(nP) is complex and variable. Analysis of the fluorescence transients observed on removal of CO_2 from pea leaves grown at 23°C demonstrate that a reduction of Q occurs together with an increase in q(nP) and substantiates the hypothesis of Krause [15] and Sivak et al. [12] that CO_2 removal results in a reduction of noncylic electron flow, with the consequent reduction in Q and a slower increase in ΔpH across the thylakoids as the major sink for ATP is removed. It should be noted that immediately on removal of CO_2 q(nP) shows a transitory decrease prior to the slow increase, which may be due to a transitory decrease in transthylakoid ΔpH as noncyclic electron flow is reduced more rapidly than ATP consumption. Analysis of a very similar fluorescence transient on depletion of CO_2 from pea leaves grown at 15°C did not support such a hypothesis. Removal of the CO_2 from the 15°C grown leaves produced an oxidation of Q and not a reduction as seen in the 23°C grown leaves, although the changes in q(nP) were similar in leaves grown at both temperatures. It is difficult at present to provide a satisfactory explanation of this difference in the changes in q(P) between leaves grown at the two temperatures. At steady-state photosynthesis prior to the removal of CO_2, Q was more reduced and q(nP) was higher in the 15°C grown leaves, possibly indicating a lower rate of noncyclic electron flow and ATP consumption and a different metabolic status of the stroma than was the case in the 23°C grown leaves. It would be expected that leaves grown at these different temperatures would have different pool sizes of key metabolites, such as phosphoglyceric acid, prior to the generation of the fluorescence transient on depletion of CO_2.

It is evident from the data presented that O_2 plays an important role in determining the nature of the fluorescence transients generated on removal of CO_2 from a leaf. In the absence of O_2 both 15°C (Fig. 5) and 23°C (data not shown) grown leaves exhibited no change in fluorescence quenching when CO_2 was removed and Q was found to be almost totally reduced at all times, implying that the overreduction of electron transport components in the absence of O_2 prevented the initiation of electron flow to CO_2. The suggestion that O_2 is required to allow the initiation of electron flow to CO_2 by acting as an electron acceptor and by releasing inhibition of cyclic electron flow [15,26] is compatible with the data presented here. In the presence of CO_2 and O_2 both noncyclic and cyclic electron transport would be expected to contribute to the ΔpH across the thylakoid membrane [30,31] which would produce a nonphotochemical quenching of fluorescence.

Oxygen can accept electrons from either PS I [24] or PS II [27]; however, on removal of CO_2 from the 15°C grown leaf the oxidation of Q would appear to be achieved by O_2 accepting electrons from an electron donor prior to B. The nature of the fluorescence transients generated on exposure of the leaf to a second excitation given at steady state prior to and after the removal of CO_2 (Fig. 3) implies that B is considerably more reduced after the removal of the CO_2, although Q is more oxidised. Oxygen may be accepting electrons directly from Q. This being the case, it would have to be argued that the slow increase in q(nP) to steady state after CO_2 removal is due to an increased ΔpH produced only from water oxidation or cyclic electron transport, since the rate of proton pumping associated with noncylic electron flow through plastoquinone would not increase. In fact, since B is becoming more reduced it can be argued that there would be a reduction in the rate of electron flow through plastoquinone. Clearly, fur-

ther experiments examining the redox state of Q, B and plastoquinone are required to substantiate such a hypothesis. The suggestion that O_2 accepts electrons from a donor before B is inconsistent with the observation that removal of O_2 has no effect on fluorescence quenching in leaves treated with DCMU (data not presented, but see Ref. 24), which implies that O_2 accepts electrons after the DCMU-binding site. However, it is possible that modification of the B-protein as a result of DCMU binding changes the ability of the PS II primary electron acceptors to reduce O_2.

This study adds additional weight to the argument that fluorescence transients contain useful information on the metabolic changes occurring within the chloroplast. However, it cannot be overemphasised that to extract such information the fluorescence transients must be carefully analysed. This point is clearly made from the comparison of the fluorescence transients generated on depletion of CO_2 from 15 and 23°C grown pea leaves (Fig. 1). Although these fluorescence transients were remarkably similar, the underlying changes in q(P) and q(nP) were considerably different demonstrating the problems that the use of the fluorescence level alone as an indicator of photosynthetic function can create.

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