Evidence for a physiological role of CO_2 in the regulation of photosynthetic electron transport in intact leaves

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The effect of CO_2 upon photosynthetic electron transport was examined in wheat and maize leaves in order to establish whether CO_2 had a direct role in electron-transport regulation in vivo. When intercellular CO_2 was depleted a transient rise in chlorophyll fluorescence occurred which correlated with an increase in the reduction of the Photosystem II primary quinone acceptor, Q_A, and a decrease in CO_2 fixation rate. However, when intercellular CO_2 was reduced from an already low level (50 μmol $\cdot$ mol^{-1}) towards zero a substantial further reduction in Q_A occurred with little change in fluorescence or CO_2 fixation. In very low intercellular CO_2 when no measurable CO_2 fixation was sustained, an appreciable fraction of Q_A still remained oxidised, however, maximal reduction of Q_A occurred when O_2 was also removed. Q_A could then be substantially reoxidised by the readdition of small amounts of CO_2 (20–40 μmol) which only facilitated a very small increase in CO_2 fixation. Changes in the kinetics of the fast rise in fluorescence emission indicated that Q_A-to-Q_B electron transfer was decreased in a CO_2-free atmosphere and Q_B was poised in a more oxidised state. Electron transport that was independent of CO_2 fixation was measured in methyl viologen-treated leaf discs. In 1% O_2, but not in 21% O_2, light-dependent electron transport to methyl viologen was decreased significantly by the depletion of CO_2. It is concluded that CO_2 can modify the redox state of Photosystem II electron transport acceptors in vivo independently of carbon assimilation and that there is a complex interaction between CO_2 and O_2 in the regulation of photosynthetic electron transport. The possibility that CO_2 operates via the reversible binding to PS II and thereby acts as a cofactor for efficient PS II electron transport in the leaf is discussed.

Abbreviations: A, rate of CO_2 fixation; C_i, intercellular CO_2 concentration; F_{680}, 680 nm chlorophyll fluorescence emission; PS I, Photosystem I; PS II, Photosystem II; Q_A and Q_B, primary and secondary quinone acceptors of Photosystem II, respectively; q(P) and q(nP), photochemical and non-photochemical fluorescence quenching, respectively.

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Introduction

The depletion of CO_2 from the atmosphere surrounding a leaf produces complex changes in the kinetics of chlorophyll fluorescence emission which are partially attributable to changes in the redox state of the PS II primary quinone electron acceptor, Q_A. In both C3 and C4 species, when CO_2 is removed chlorophyll fluorescence rises

rapidly but is then quenched back to a level little different to that preceding CO_2 depletion [1–3]. At the same time Q_A usually becomes increasingly, although never totally, reduced [3,4]. Furthermore, a complex interaction between CO_2 and O_2 occurs. The removal of CO_2 from O_2-free air induces a complete but reversible reduction of Q_A and a large sustained rise in fluorescence emission [3,5]. These effects of CO_2 and O_2 concentration upon the redox state of acceptors and the rate of electron transport have been considered previously to be exerted solely through a feed-back reaction from changes in rates of carbon assimilation [3,5].

The possibility that CO_2 exerts a more direct effect upon PS II electron transport is evoked by the observation in vitro that maximum rates of PS II electron transport are dependent upon the binding of CO_2, probably in the form of bicarbonate ion [6], to PS II. The site of binding has not been specifically identified but is certainly located on the reducing side of PS II and is likely to be close to the Q_B protein [7]. Binding of CO_2/HCO_3^- to PS II facilitates exchange of Q_B-bound quinol with non-bound platoquinone [8–10]. At low HCO_3^- concentrations other species, particularly formate, compete for the anion binding site and inhibit PS II electron transfer [10–14]. These observations raise the question of whether CO_2 has an important regulatory role in photosynthetic electron transport. It has been speculated previously that the binding of CO_2 links electron transport with cell metabolism and affects the NADP/ATP ratio in the chloroplast [13]. However, there is no experimental evidence at the present time that reversible association of HCO_3^-/CO_2 with PS II occurs when the CO_2 concentration inside the leaf varies causing a direct effect on the redox state of PS II electron acceptors and PS II electron-transport rate in vivo.

The aim of this paper is to evaluate the role of CO_2 in photosynthetic electron transport in intact leaves. The effect of CO_2 on a number of parameters of photosynthetic electron transport is examined in vivo. CO_2-induced changes in the kinetics of 680 nm chlorophyll fluorescence emission, which emanates primarily from PS II [15], are studied in detail and used to monitor the redox state of the PS II quinone electron acceptors. Changes in the CO_2 fixation rate and the leaf

intercellular concentration of CO_2 are determined and related to the changes in fluorescence emission. Non-cyclic electron transport that is independent of carbon assimilation, is examined by measuring O_2 exchange in leaf discs infiltrated with methyl viologen. In such tissue methyl viologen replaces CO_2 as the terminal electron acceptor and any observed effects of CO_2 can then be attributed to a direct effect on electron transport rather than to CO_2 acting as the terminal acceptor. The evidence presented supports the hypothesis that CO_2 acts as a cofactor for Photosystem II electron transport in intact leaves.

Materials and methods

Plant material. Wheat (*T. aestivum* L. cv Maris Dove) and maize (*Z. mays* L. cv LG11) plants were grown in a growth cabinet at $23°C$. Photosynthetically active radiation was supplied at 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during 16 h photoperiods. For CO_2 exchange and chlorophyll fluorescence measurements sections of 6 cm in length were cut from the distal mature region of leaves of either 2-week-old maize or 3-week-old wheat plants. For measurement of O_2 exchange leaf discs were cut from the mid-region of 4-week-old maize plants.

Measurement of leaf gas exchange and estimation of intercellular CO_2 concentration (C_i). Wheat or maize leaf sections were sealed in a temperature-controlled leaf section chamber ($23°C$; ADC Ltd., U.K.) with their cut ends held outside the gas-exchange compartment of the chamber and immersed in circulating deionised H_2O. N_2, O_2 and CO_2 were mixed at controlled rates using thermal mass flow controllers (FC-260, Tylan Inc.) and humidified using a water vapour generator (WG600, ADC Ltd.). Half the gas flow was passed through the leaf chamber at 5 $cm^3 \cdot s^{-1}$ and half by-passed the chamber to create a reference flow. The humidity difference across the leaf chamber was measured with electrical capacitance humidity probes (Humilab, Lee Integer, U.K.). Illumination was supplied as described below. The absolute and differential CO_2 concentrations were measured with two infrared gas analysers (LCA and Series 225/3, ADC Ltd.) placed in series. The rate of CO_2 fixation by the leaf (A) and stomatal conductance (g_s) were determined using the standard

equations for an open gas-exchange system [16]. Intercellular CO_2 concentration (C_i) was then estimated from the calculated assimilation rate and leaf conductances using the equations of Von Caemmerer and Farquhar [17].

Measurement of 680 nm chlorophyll fluorescence emission (F_{680}). The experimental system employed was a modification of that previously described [2,3]. Following 30 min darkness, leaf sections were irradiated with light from a quartz-iodide source passed through a heat-reflecting mirror (Ealing Beck Ltd., U.K.) and a cut-off filter (less than 580 nm transmission; 35-388, Ealing Beck) giving a photon flux density at the sample surface of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Chlorophyll fluorescence emission was transmitted by a fibre optic from the leaf to a photomultiplier tube (Hamamatsu type R446, Hakuto International, U.K.) protected by a 680 nm interference filter (35-4068, Ealing Beck). Analogue signals from the

gas analysers and photomultiplier were fed to a microcomputer via a 12-bit A–D converter (PCI 6300, CIL Microsystems Ltd., U.K.) for analysis. In addition, the photomultiplier signal was sent to a digital storage oscilloscope (type 142, Gould Inc.) for measurement of the fast transients in chlorophyll fluorescence emission.

Estimation of the redox state of the PS II primary electron acceptor, Q_A. The redox state of Q_A was estimated by a technique established previously [18–20]. An illuminated leaf section was excited by a second irradiation of saturating intensity (1000 μmol photons for 2 s) and the rapid rise in fluorescence generated was monitored with a digital storage oscilloscope. The ratio of the new variable fluorescence generated, F_{V2}, to the new minimal level, F_{O2}, estimates the redox state of Q_A, i.e., a value for F_{V2}/F_{O2} of zero indicates maximal reduction of O_A and increasing values indicate increasing amounts of oxidation of Q_A. It

Fig. 1. The response of 680 nm chlorophyll fluorescence emission ($F680$), the rate of CO_2 fixation (A), and F_{V2}/F_{O2}, which estimates the redox state of Q_A, to changes in the intercellular CO_2 concentration (C_i) in the wheat leaf. Steady-state photosynthesis was induced in 30 min dark-adapted leaf sections by a photon flux density of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in 350 $\mu mol \cdot mol^{-1}$ CO_2 and 20% O_2 (panel a). The external CO_2 concentration was subsequently controlled in order to modify C_i (panels b, c). F_{V2}/F_{O2} was measured from the fast rise in fluorescence generated by an additional 2 s photon flux density of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

should be noted that although the variable fluorescence is closely related to the reduction state of Q_A, a strict linear relationship between the two does not exist.

Measurement of O_2 exchange by methyl viologen-treated leaf discs. Maize leaf discs (area 10.75 cm^2) were immersed in 1 mM methyl viologen containing 0.1% Tween 20 for 4 h in the dark. The surfaces of the disc were dried rapidly and the disc transferred to a temperature-controlled leaf disc O_2 electrode chamber (Hansatech Ltd., U.K.). The electrode was calibrated and the rate of O_2 exchange measured at 20°C according to the method of Delieu and Walker [21]. White light was provided by a projector lamp giving a photon flux density at the leaf surface of 500 μmol · m^{-2} · s^{-1}. CO_2-free air was produced by enclosing a few granules of soda lime in the chamber and a 0.1 M bicarbonate-carbonate buffer was employed to produce external CO_2 concentrations between zero and 500 μmol · mol^{-1}. O_2 was supplied by flushing the chamber from either a 1% or 21% O_2 source. After a steady-state rate of O_2 uptake was achieved

in the dark, O_2 exchange was measured in the light, typically for 20 min, and the dark rate then remeasured. Immersion of leaf discs in solution without methyl viologen provided an appropriate control treatment.

Results

Effect of depletion of intercellular CO_2 at steady-state photosynthesis on 680-nm chlorophyll fluorescence emission, carbon assimilation rate and redox state of Q_A

In both wheat and maize leaves the complete removal of CO_2 from the atmosphere caused a transient rise in 680-nm chlorophyll fluorescence emission which correlated closely in time with a decrease in the rate of CO_2 fixation (Figs. 1 and 2) as described previously [1,3]. A previous analysis [4] has shown that this transient correlates with a decrease in photochemical quenching, $q(P)$, resulting from the reduction of Q_A, followed by an increase in non-photochemical quenching, $q(nP)$, due presumably to the fall in consumption of ATP
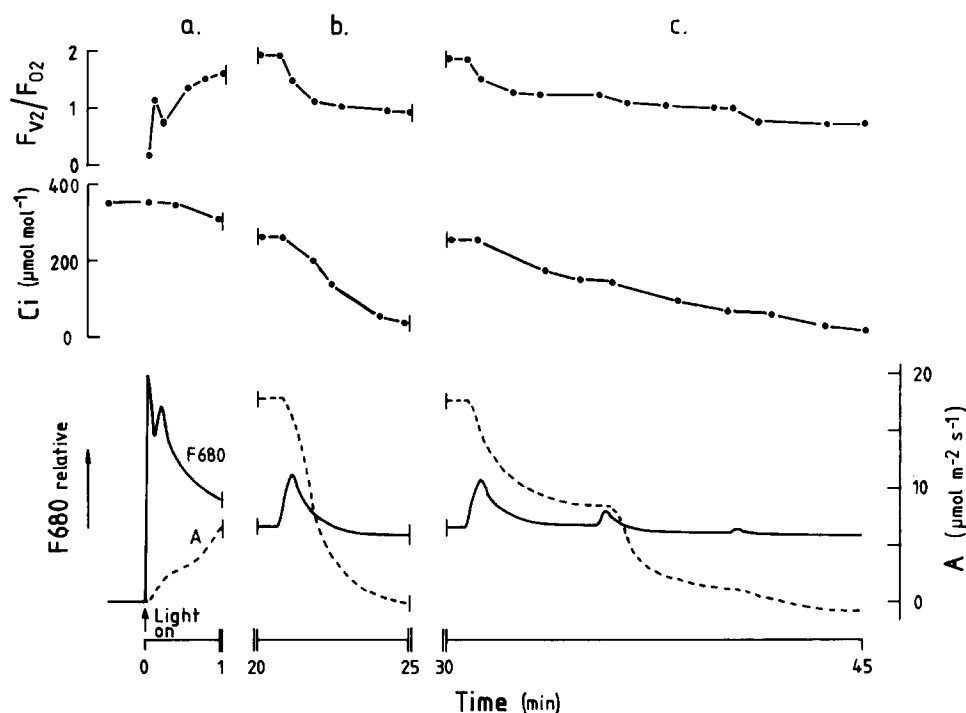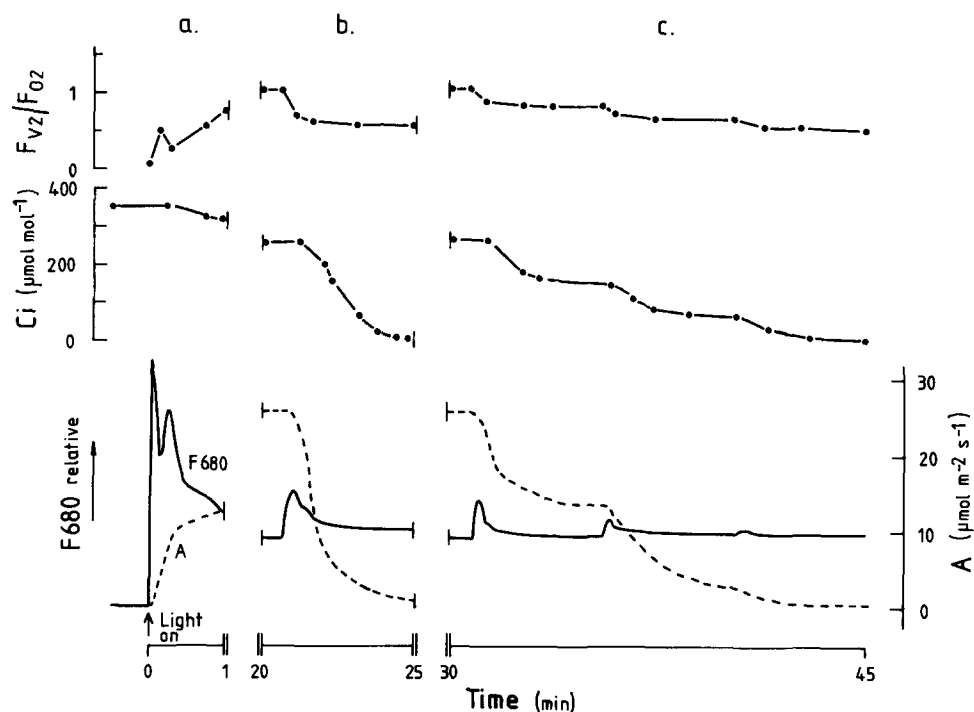

Fig. 2. The response of 680 nm chlorophyll fluorescence emission ($F680$), rate of CO_2 fixation (A), and F_{V2}/F_{O2}, which estimates the redox state of Q_A, to changes in the intercellular CO_2 concentration (C_i) in the maize leaf. Experimental conditions and method are as in Fig. 1.

and a consequential rise in phosphorylation potential, ΔG_{ATP}, and *trans*-thylakoid ΔpH.

The external CO_2 level was altered in order to manipulate the intercellular CO_2 concentration (C_i) and the effect on chlorophyll fluorescence (F_{680}), CO_2 fixation rate (A) and the redox state of Q_A were measured. When C_i, in both wheat and maize leaves, was decreased from 250 to 100 μmol $\cdot$ mol^{-1} (Figs. 1 and 2; panel c) a transient in F_{680} occurred which was almost as large as that produced when C_i was decreased from 250 to 20 μmol $\cdot$ mol^{-1} in wheat (Fig. 1; panel b) and from 250 to near zero μmol $\cdot$ mol^{-1} in maize (Fig. 2; panel b). A further decrease of C_i from 100 to 50 μmol $\cdot$ mol^{-1} produced only a very small F_{680} transient in both species and a still further depletion towards zero had a negligible effect (Figs. 1 and 2; panel c). All of the fluorescence transients correlated in time with the decrease in A.

The depletion of intercellular CO_2 in wheat and maize leaves towards zero caused a decline in F_{V2}/F_{O2}. The reduction state of Q_A is not linearly related to variable fluorescence but rather to the area above the fluorescence curve [22,23]. Although the quantitative relationship between F_{V2}/F_{O2} and the reduction state of Q_A has not been determined, the decrease in F_{V2}/F_{O2} observed must represent a considerable reduction of Q_A. However, Q_A was not maximally reduced by this treatment, as F_{V2}/F_{O2} did not fall to zero. As in the case of the transient rise in F_{680}, a large reduction of Q_A was dependent upon a large absolute change in C_i. Upon reduction of C_i from 50 μmol $\cdot$ mol^{-1} towards zero a measurable change in F_{V2}/F_{O2} occurred although only a very small transient in F_{680} was produced. This suggests that at low levels of C_i (less than 50 μmol $\cdot$ mol^{-1}) changes in the redox state of Q_A can take place which have little effect on chlorophyll fluorescence emission and, considering the low CO_2 fixation rate, may be independent of carbon assimilation. As shown in Figs. 1 and 2, there were no obvious differences between C4 maize and C3 wheat in this respect.

The effect of CO_2 concentration upon the redox state of Q_A in O_2-free air

In an anaerobic atmosphere, the removal of CO_2 from the wheat leaf caused an initial tran-

Fig. 3. (A) The response of 680 nm chlorophyll fluorescence emission ($F680$) and F_{V2}/F_{O2}, which estimates the redox state of Q_A, to changes in the external CO_2 concentration in the wheat leaf at steady state in O_2-free air. Photon flux density was 500 μmol$\cdot$m$^{-2}\cdot$s^{-1}; CO_2 concentrations (μmol$\cdot$mol^{-1}) are shown in parentheses. (B) F_{V2}/F_{O2} as a function of the external CO_2 concentration in wheat and maize leaves in O_2-free air.

sient rise in F_{680} which was quickly followed by a large and sustained rise in emission (Fig. 3A). The transient rise appears similar to that produced by the removal of CO_2 in a normal atmosphere, while the sustained rise correlated with a continuing decrease in F_{V2}/F_{O2}. In maize a similar sustained rise in fluorescence occurred (data not shown) although it was not as large as that in wheat. In both species, F_{V2}/F_{O2} values indicated that Q_A eventually became maximally reduced. The readdition of CO_2 caused F_{680} to be quenched back to its original level and Q_A to be reoxidised.

Experiments in which CO_2 was completely withdrawn from an O_2-free atmosphere surrounding the leaf and then re-introduced in small

aliquots indicated that in wheat a re-introduction of 10 μmol $\cdot$ mol^{-1} CO_2 caused only a very slight quenching of F_{680} and negligible change in F_{V2}/F_{O2} (Fig. 3A). An addition of 30 μmol $\cdot$ mol^{-1} CO_2, however, caused a very large quenching of F_{680} and a large increase in F_{V2}/F_{O2}, while a further addition to 75 μmol $\cdot$ mol^{-1} CO_2 caused an almost complete quenching back to the original level of emission and F_{V2}/F_{O2} increased to the original value. Further additions of CO_2 had no effect on F_{680} or F_{V2}/F_{O2}. Very similar results were obtained in maize and in Fig. 3B F_{V2}/F_{O2} is plotted against CO_2 concentration (in an O_2-free atmosphere) for both wheat and maize; the observed rates of CO_2 fixation during the experiment are also shown. In both species half the
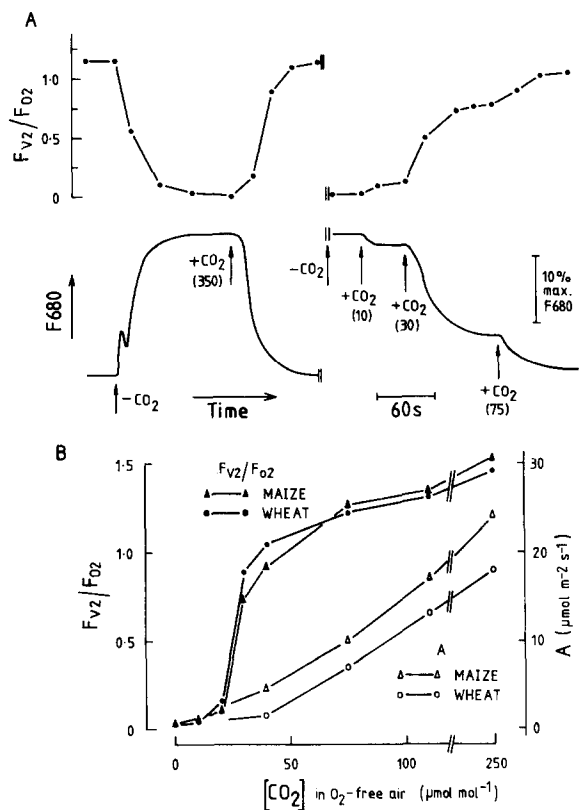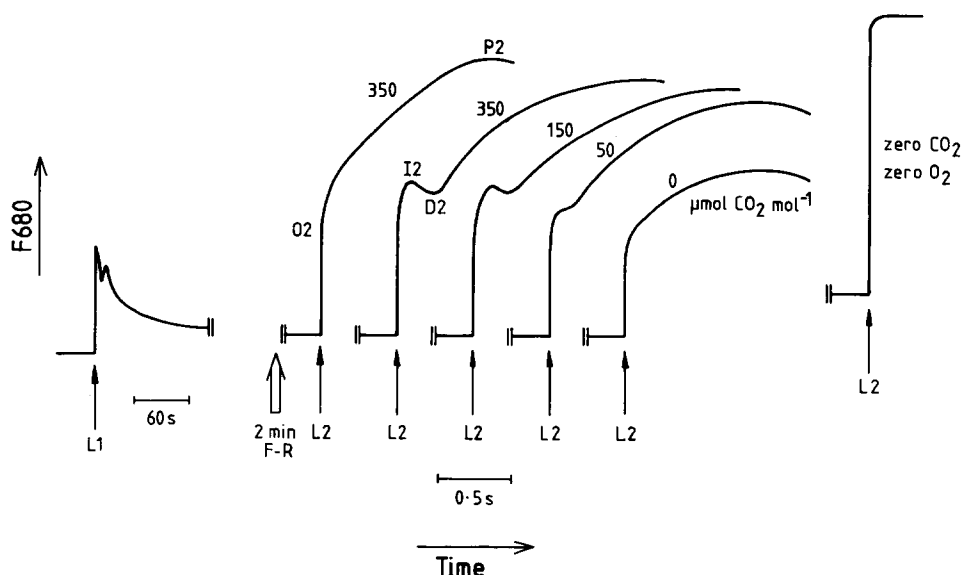

Fig. 4. (A) The response of 680 nm chlorophyll fluorescence emission ($F680$) and F_{V2}/F_{O2}, which estimates the redox state of Q_A, to changes in the external O_2 concentration in the wheat leaf at steady state in CO_2-free air. Photon flux density was 500 μmol$\cdot$m$^{-2}\cdot$s^{-1}; O_2 concentrations (%) are shown in parentheses. (B) F_{V2}/F_{O2} as a function of the external O_2 concentration in wheat and maize leaves in CO_2-free air.

maximum F_{V2}/F_{O2} value is attained by the addition of approx. 30 μmol $\cdot$ mol^{-1} CO_2. At this level of CO_2, C_i in both wheat and maize leaves is very low and sustains little CO_2 fixation. In the absence of O_2, the intercellular CO_2 concentration required to saturate A under the same light level was found to be 225 μmol $\cdot$ mol^{-1} in wheat and 215 μmol $\cdot$ mol^{-1} in maize. Clearly, the large effect of the re-addition of small amounts of CO_2 on the redox state of Q_A is unlikely to be caused through changes in the rate of carbon assimilation. Similar results were also obtained with pea leaves (data not shown).

The effect of O_2 concentration upon the redox state of Q_A in CO_2-free air

It is clear from results described both above and previously [3,4]) that the effect of CO_2 upon the redox state of PS II electron acceptor is modified extensively by the availability of O_2. In order to examine this further, similar types of experiments to those described above were undertaken in which O_2 was withdrawn from wheat and maize leaves in a CO_2-free atmosphere and re-introduced at various concentrations. The results are shown in Fig. 4A and B. Upon removing all the O_2 from a leaf in CO_2-free air, F_{680} rapidly rose to a high and sustained level and Q_A became totally reduced, i.e., F_{V2}/F_{O2} decreased to zero. The subsequent reintroduction of 2% O_2 had a negligible effect on the measured parameters. However, in wheat, F_{680} was significantly quenched and Q_A reoxidised by the reintroduction of 3% O_2 and a complete quenching was produced by 5% O_2 (Fig. 4B). The effect in maize was similar, although slightly higher concentrations of O_2 were required, i.e., 3.5% O_2 required to produce 50% requenching and significant reoxidation of O_A (Fig. 4B).

The effect of CO_2 on the fluorescence I–D transient generated in leaves at steady state photosynthesis

Upon irradiating a dark-adapted leaf, the fast rise in fluorescence from the minimal level, F_O, to the peak, F_P, contains a transient with a shoulder and/or dip in emission commonly occurring after about 200 ms. This is referred to as the I–D transient and is attributed to the reoxidation of Q_A^- by Q_B, the secondary quinone acceptor of PS II [24,25]. The transient is enhanced by pre-il-

Fig. 5. The kinetics of 680 nm chlorophyll fluorescence emission ($F680$) upon addition of a second irradiation (L2, 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to the wheat leaf following (i) a 2 min irradiation with 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of far-red light (710 nm), (ii) a 5 min exposure to air containing different levels of CO_2, (iii) a 5 min exposure to O_2-free and CO_2-free air. The 30 min dark-adapted leaf was initially supplied with a photon flux density of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (L1) in air containing 350 $\mu mol \cdot mol^{-1}$ CO_2 and 20% O_2. The points O2, I2, D2 and P2 represent standard points on the fluorescence induction curve as discussed in the text.

lumination of leaves with blue light, which preferentially excites PS II and poises PS II acceptors in a relatively reduced state so that subsequent Q_A^- to Q_B electron transfer is slow [4,25,26]. A similar I–D transient was found to be generated by exposing a leaf, which had already attained steady-state photosynthesis at one light level, to a second high-intensity irradiation (Fig. 5). This transient was abolished when the leaf was exposed to an additional far-red light (710 nm) for 2 min immediately prior to the second high light, and can be considered analogous to the I–D transient generated in dark-adapted tissue. The I–D transient is predicted to be large when Q_B is poised in a relatively reduced state and PS II electron transfer is slow.

Fig. 5 shows that the complete removal of CO_2 from wheat leaves 5 min before the addition of the second light caused the I–D transient to be lost whereas low concentrations of CO_2 close to the CO_2 compensation point (50 $\mu mol \cdot mol^{-1}$) allowed it to be retained. A similar result was obtained in maize where the CO_2 concentration was reduced to 75 $\mu mol \cdot mol^{-1}$ before the I–D transient was diminished (data not shown). In both

species, with CO_2 present, the I–D transient was insensitive to oxygen concentration (data not shown). These results suggest that in a leaf at steady-state photosynthesis the removal of CO_2 causes Q_B to be poised in a more oxidised state than would be the case in the presence of CO_2, thus removing the possibility of generating an I–D transient by exposure to a second high intensity excitation.

The effect of CO_2 concentration upon O_2 exchange rate in methyl viologen-treated maize leaf discs

In control leaf discs held in 21% O_2 and 350 $\mu mol \cdot mol^{-1}$ CO_2 there was a steady-state rate of net O_2 evolution of 22.5 ± 3.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the light (photon flux density 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) which became 23.8 ± 4.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when O_2 was decreased to 1%. Fig. 6 shows that in methyl viologen-treated maize leaf discs light-dependent O_2 evolution was prevented and there was instead a predicted light-promoted net consumption of O_2. This indicated that methyl viologen had replaced CO_2 as the terminal electron acceptor in the leaves and subsequent oxidation of reduced methyl viologen by molecular O_2 resulted
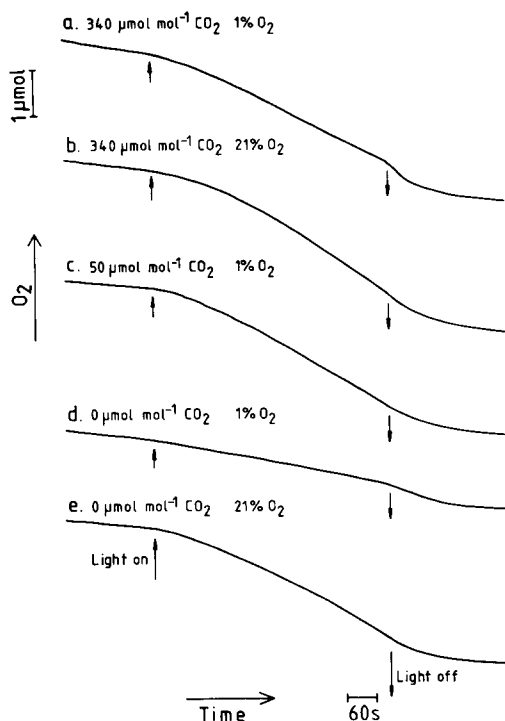
Fig. 6. Oxygen exchange by methyl viologen-treated maize leaf discs. Leaf discs were immersed in the dark in 1 mM methyl viologen for 4 h and O_2 exchange then measured in a leaf disc O_2 electrode chamber in different CO_2 levels with either 21% or 1% O_2. Photon flux density was 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Steady-state rates of O_2-exchange rates achieved in the light were as follows; (a) -4.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$; (b) -5.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$; (c) -4.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$; (d) -1.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ($P < 0.05$); (e) -4.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Leaf discs immersed in solution lacking methyl viologen supported a mean O_2-evolution rate of $+23.8 \pm 4.4$ $\mu mol \cdot m^{-2}$ when held under the same conditions.

in a net uptake of O_2 [27,28].

Fig. 6 shows the results of one typical experiment in which the effect of CO_2 and O_2 concentration upon the O_2 exchange rate in methyl viologen-treated leaves is demonstrated. When O_2 was present in the electrode chamber at a level of 21%, the rate of O_2 consumption in the light by methyl viologen-treated leaf discs was not significantly affected by alteration of the CO_2 supply. In an atmosphere containing 1% O_2, however, removal of CO_2 caused a significant decrease in the rate of O_2 consumption of around 66% compared to the leaf disc held in a normal atmosphere (Fig. 6). An enhanced O_2 consumption rate in the light could be restored by increasing either the CO_2

level to 50 $\mu mol \cdot mol^{-1}$ or flushing with 21% O_2, but not necessarily both. These results indicate that in the presence of low amounts of O_2, CO_2 is required to facilitate whole chain photosynthetic electron transport to methyl viologen in vivo.

Discussion

The depletion of CO_2 from a leaf at steady state photosynthesis would be expected to produce changes in both the redox state of Q_A and the transthylakoid ΔpH [29]. However, in both wheat and maize, a small decline from an already very low level of intercellular CO_2 caused a significant reduction of Q_A while producing only a small decrease in CO_2 fixation and a negligible transient in fluorescence emission (panel c of Figs. 1 and 2). Thus, the observed change in Q_A redox state at low C_i appeared to be independent of carbon assimilation. The negligible effect on F_{680} was presumably due to the high ΔpH which would exist in the CO_2-depleted thylakoids and which would produce a high level of non-photochemical fluorescence quenching prior to further depletion of CO_2. It has been shown previously that under such conditions non-photochemical quenching is by far the largest factor determining fluorescence emission and small changes in photochemical quenching, $q(P)$, created by changes in the redox state of Q_A, have little effect on fluorescence emission [4].

The removal of CO_2 from O_2-free air produced a maximal reduction of Q_A (Fig. 5A and B, Refs. 3–5). Reoxidation of Q_A under these conditions was induced by the readdition of only small amounts of CO_2. Approx. 66% recovery of variable fluorescence was brought about in both wheat and maize leaves by an increase in external CO_2 from only 20 to 40 $\mu mol \cdot mol^{-1}$ (Fig. 3A and B), which produced only very small increases in CO_2 assimilation rate, i.e., approx. 1.5% and 2.5% of maximum rates in wheat and maize, respectively. Clearly changes in the rate of carbon metabolism are extremely unlikely to account for the marked reoxidation of Q_A.

The loss of the I–D transient in fluorescence emission when irradiated leaves were depleted of CO_2 and exposed to an additional saturating light (Fig. 4) suggests that under these conditions Q_A^- to

Q_B electron transfer has been decreased. A direct effect of CO_2 upon PS II in vivo, which is suggested by this observation, was confirmed by the decrease in light-dependent electron transport in methyl viologen-treated leaves (Fig. 5). Methyl viologen replaced CO_2 as the terminal electron acceptor and thus the effect of CO_2 cannot be attributed to changes in CO_2 assimilation. There was, however, an interaction between CO_2 and O_2 in mediating this effect. The decrease in electron transport induced by lowering the CO_2 concentration only occurred in low O_2 tensions, implying that O_2 acts as an alternative electron acceptor when CO_2 becomes unavailable.

It is possible that the redox state of PS II acceptors is modified in vivo by the reversible binding of CO_2/HCO_3^- to the PS II complex at a site close to Q_B. The low concentrations of CO_2 that are active in modifying F_{V2}/F_{O2} values are consistent with this idea, since the CO_2/HCO_3^- binding site has a low dissociation constant in vitro of 70–100 μM which is increased to about 1.3 mM HCO_3^- in the presence of 100 mM formate [11–14]. From the observed values of intercellular CO_2 concentration, the liquid CO_2 concentration can be calculated and the level of intercellular bicarbonate then predicted from the Henderson-Hasselbach equation. The physiological concentration of HCO_3^-/CO_2 in the chloroplast has been reported to be as low as 5 μM in the light [11,30]. While this would approximate to the concentration of CO_2 in solution, this analysis does not take account of the larger pool of HCO_3^- which must exist at equilibrium. Using a value of 6.11 for the dissociation constant (pK) of H_2CO_3 in the chloroplast (at 20°C and ionic strength 150 μM; Ref. 31), our calculations suggests that under normal atmosphere conditions (330 μmol CO_2 per mol) when C_i is about 230 $\mu mol \cdot mol^{-1}$ in C_3 species and stromal pH is assumed to be 8 in the light, the HCO_3^- concentration would be approx. 500 μM. This agrees with the findings of Blubaugh and Govindjee [6] and it is concluded that under normal conditions in the chloroplast HCO_3^- is bound to PS II. This analysis would need to be revised if it were shown that the HCO_3^- binding site resided entirely on the inside of the thylakoid where a low pH prevails. Although it has been suggested that it is intra-thylakoid pH that affects

binding [31], this study did assume CO_2 rather than HCO_3^- to be the actively bound species.

Based on the above analysis it is predicted that at the levels of CO_2 which were effective in modifying the redox state of Q_A, i.e., 20–30 $\mu mol \cdot mol^{-1}$, chloroplast HCO_3^- would fall to concentrations in the order of 10 μM at which dissociation of HCO_3^- from PS II would occur. Furthermore, even if the experimental depletion of external CO_2 is unable in itself to facilitate the dissociation of HCO_3^- from PS II, the competition for the common anion binding site with formate and other anions [11,13,33] may increase HCO_3^- removal from PS II when low levels of CO_2 prevail at the chloroplast. While this is only speculation, it has been shown previously that in the presence of formate the CO_2/HCO_3^- binding constant is 3-fold greater in the light than in the dark [11] implying that dissociation is enhanced in the light. This might also explain why CO_2 has no apparent effect on the I–D transient generated in dark-adapted leaves, i.e., the transient occurs in the first 200 ms after excitation which is prior to any light-favoured dissociation of HCO_3^- taking place. We therefore suggest that the reversible binding of HCO_3^-/CO_2 to PS II has a role in the regulation of the redox state of PS II quinone acceptors in vivo when CO_2 is depleted in the leaf.

In conclusion, the data presented indicate that CO_2 has a direct effect on the redox state of PS II electron acceptors and acts as a cofactor of photosynthetic electron transport. However, when CO_2 supply is low molecular O_2 can act as an alternative acceptor, maintaining Q_A in a partially oxidized state and facilitating electron-transport activity. The fact that the response of the Q_A redox state to alterations in O_2 supply were similar in C3 wheat leaves and C4 maize leaves and that similar changes occur in immature tissue in which RuBP carboxylase/oxygenase is inactive (3) demonstrate that photorespiration cannot be the major process mediating the oxygen effect. It may thus be speculated that when CO_2 concentration at the chloroplast is low, a dissociation of CO_2/HCO_3^- from PS II (and possibly its replacement by formate) inhibits electron transport from Q_A, through Q_B, to PS I. However, under these conditions oxygen can act as an electron acceptor and prevent the over-reduction of PS II quinone

acceptors. It is possible that in this way the plant can cope with a situation in which there is limited CO_2 available to act as a terminal acceptor for photosynthetic electron transport.

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