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A long-lasting photorespiration in CO_2-free air, measured as the postirradiation CO_2 burst, indicates mobilization of storage photosynthates

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Abstract

In CO_2-free air, the CO_2 postirradiation burst (PIB) in wheat leaves was measured with an IRGA in an open gas exchange system to ascertain its potential role in alleviating photoinhibition of photorespiratory carbon oxidation (PCO) under a CO_2 deficiency. A pre-photosynthesized leaf having been transferred into CO_2-free air exhibited a typical CO_2 PIB following darkening which could last, with a rate substantially higher than that of dark respiration, over a long time period (at least more than 2 h) of continuously alternate irradiation (2 min)-dark (2 min)-light transitions. The rate and the time of PIB maintenance, although unaffected by the exogenous dark respiration inhibitor iodoacetic acid, were stimulated largely by increasing irradiance and O_2 level, and suppressed by DCMU and N-ethyl-maleimide (NEM). They also showed a large photosynthates-loading dependence. In a darkened leaf, the irradiation-induced PIB in the CO_2-free air was clearly revealed and it was characterized by an initial net uptake of respiratory CO_2. The light-induced PIB was accelerated by increasing irradiance, and delayed by prolonging the period of darkening the leaves. Hence, the origin of carbon needed for a long-term CO_2 evolution in the CO_2-free air might not only be derived directly from the pool of intermediates in the Calvin cycle, but it might also arise indirectly from a remotely fixed reserve of photosynthates in the leaf *via* a PCO-mediated, yet to be further clarified, mobilization process. Such mobilization of photosynthates probably exerted an important role in coordination of photochemical reactions and carbon assimilation during photosynthesis in C_3 plants under the photoinhibitory conditions.

Additional key words: DCMU; iodoacetic acid; N-ethylmaleimide; photoinhibition; P_i; *Triticum*.

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Abbreviations: DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; DHAP, dihydroxyacetone phosphate; IRGA, infrared gas analyzer; NEM, N-ethylmaleimide; PAR, photosynthetically active radiation; PCO, photorespiratory carbon oxidation; PCR, photosynthetic carbon reduction; PIB, CO_2 postirradiation burst; RuBPCO, ribulose 1,5-bisphosphate carboxylase/oxygenase.

Introduction

Relatively high values of CO_2 compensation concentration and O_2-sensitive net carbon fixation are distinguishable photosynthetic gas exchange characteristics among various C_3 plants (Edwards and Walker 1983, Šesták 1985). In addition, when an irradiated C_3 plant leaf is plunged into darkness, CO_2 is initially released at a high rate and then it drops to a concentration associated with the normal dark respiration. This phenomenon was observed and termed as CO_2 postillumination (postirradiation) burst (PIB) by Decker (1955). CO_2 released during the CO_2 PIB mainly originates from glycollate, the primary metabolite of PCO, produced by the oxygenolytic cleavage of RuBP catalyzed by RuBPCO (Lorimer 1981, Tolbert 1983, Ogren 1984). A short-term (lasting 3-5 min) PIB in CO_2-free air, as monitored in most previous reports, is attributed to the exhaustion of intermediates in the glycollate pathway and the unavailability of its recovery in the dark (Zelitch 1968, Peterson 1983b, Sharkey *et al.* 1986, Palovský and Hák 1988, Pärnik and Keerberg 1995). Hereby we report that with a specific alternate light-dark transitions approach, the CO_2 PIB in wheat leaves in CO_2-free air can be maintained for more than 2 h. Since net photosynthetic carbon acquirement is virtually blocked in the CO_2-free air, our results indicate that remotely fixed photosynthates can be mobilized and they re-enter the Calvin cycle *via* the PCO-mediated pathway under CO_2 deficiency at a high irradiance.

Materials and methods

Plants: Seeds of wheat (*Triticum aestivum* L. cv. Yangmai No. 5) were germinated in pots (28 cm in diameter and 40 cm high) filled with a mixture of soil, peat, and vermiculite (1:1:1) and containing fertilizer (25 % N, 20 % P_2O_5, and 20 % K_2O, 5 g per pot). After seedling emergence, four to five seedlings were retained per pot. The plants were grown outdoors and watered daily. Only attached healthy flag leaves of about 5-month-old wheat plants were used for gas exchange measurements. Occasionally, other C_3 plants, rice (*Oryza sativa* L. cv. Xiunyiu No. 2), pea (*Pisum sativum* L.), and soybean (*Glycine max* L.) were also used for measurements: the results were functionally similar to those observed with wheat leaves. In this report, therefore, only the results of wheat leaves are presented.

Gas exchange measurements: Non-steady state CO_2 exchange was measured following Peterson (1983a), with an infra-red gas analyser (IRGA, *QGS-08, Beijing Analytical Instrument Factory*) in an open system as previously described by Ludwig and Canvin (1971). A special attention was paid to reducing the volume of the inlet tubing (0.4 cm in diameter and 25 cm in length) connecting the leaf chamber (4×8.5× 1 cm) with the IRGA measuring cell. A system of valves permitted a convenient check of IRGA's zero *via* bypassing the chamber by CO_2-free air. A drying mini-tube (7.0×0.8 cm) filled with calcium chloride connected the outlet tubing of the chamber with the inlet tubing of IRGA; it was regularly replaced (every 2 h) in order to eliminate interruption of water vapour flow. A preliminary test demonstrated that

such a system with 8.9 cm^3 s^{-1} of flow rate allowed IRGA to show a detectable electronic signal output within a period of less than 7 s as a response to the change of CO_2 concentration in the chamber, which was crucial for an accurate measurement of transient maximum rate of the PIB (Peterson 1983a, Zelitch 1984). CO_2-free gas mixtures containing the desired O_2 concentrations (see the text) were prepared by mixing O_2 with N_2 from commercially available cylinders. The O_2 concentration was monitored by a *CY-240* Oxygen Meter (*Shanghai Analytical Instrument Factory*). PAR was provided by a slide projector equipped with a tungsten lamp (100 W), filtered through 2 % $CuSO_4$ (m/v) solution as a heat filter. Irradiance was measured using a *LiCOR* photometer (model *LI-189*). The leaf stomata conductance was monitored with a *LI-6200* portable photosynthesis system (*LiCOR*, Lincoln, Nebraska, USA).

Chemical reagents' treatment: The wheat tillers attached with leaves were excised from the first node at the plant base immersed in distilled H_2O. The excised tillers were immediately inserted in a solution containing the respective chemical reagent. Prior to CO_2 exchange measurement, the excised tillers were irradiated with PAR of 250 $\mu mol\ m^{-2}\ s^{-1}$ for 3 h.

Determination of storage photosynthates: At time intervals during the day or during darkening the plants (see the text), the leaf discs (*ca.* 0.08 g) from five selected wheat plants were immediately frozen in liquid nitrogen and then extracted with 85 % hot ethanol. The supernatant was used for sucrose quantification according to the method of Cardini *et al.* (1955) while the particulate fraction was further hydrolyzed and assayed for starch determination following Ching *et al.* (1984). All measurements were carried out with three or more replications.

Results

The pattern of long-term CO_2 exchange in wheat leaves in CO_2-free air with alternate light-dark transitions: A typical pattern of CO_2 exchange in a pre-photosynthesized wheat leaf in CO_2-free air with alternate light-dark transitions is depicted in Fig. 1. The pulse of CO_2 evolution after darkening the leaf caused an abrupt rise of CO_2 concentration. At point *b* (see the inset in Fig. 1), a PIB peak was achieved, which denoted the transient maximum rate of PIR. Afterwards, the CO_2 release decreased rapidly over time, and finally, after about 3-4 min, the rate of dark respiration was approached at point *c*. The differences *b-b'*, *d-d'*, and *f-f'* are measures of transient maximum rate of PIB, net uptake of (dark) respiratory CO_2, and CO_2 release in the light, respectively. The PIB by a pre-irradiated leaf in the CO_2-free air could sustain more than 2 h with continuously alternate light-dark transitions, with the rate much higher than that of dark respiration (line *b'f'* in the inset in Fig. 1), although it declined progressively over the period.

Compared to the pre-irradiated one, the darkened wheat leaf exhibited a light-induced process of PIB which was characterized by an initial net uptake of respiratory CO_2 rather than by CO_2 evolution. During the initial light-dark

transitions, no detectable CO_2 PIB and/or CO_2 release in the light were monitored (Fig. 2). Instead, the leaf showed a relatively strong net uptake of respiratory CO_2.

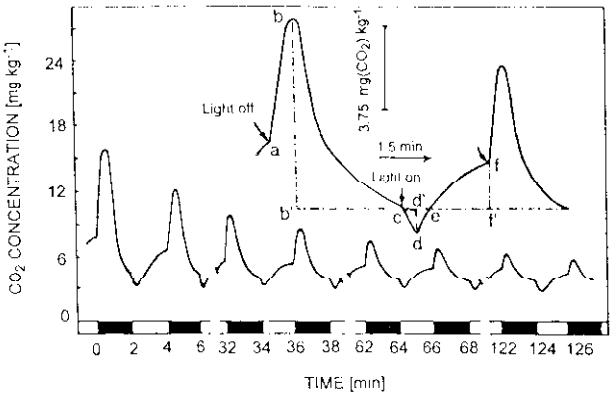

Fig. 1. The long-term CO_2 PIB by a pre-photosynthesized wheat leaf in the CO_2-free air. The leaf was irradiated in the normal air [350 mg(CO_2) kg^{-1}, 21 % O_2] for 2 h under a saturating irradiance, the air stream was then rapidly switched to the CO_2-free air, and the PIB was monitored over the period of alternate light (2 min, 850 μmol m^{-2} s^{-1})-dark (2 min) transitions (shown by the broken bar at the bottom). A typical PIB curve is also shown in the *inset* for analysis of various CO_2 exchange parameters (for details, see the text). Unless otherwise indicated, the pre-photosynthesis of the leaf in this study was always accomplished by saturation irradiation of the leaf (850 μmol m^{-2} s^{-1}) in the normal air for 2 h.

However, with the extension of alternate light-dark transitions the CO_2 PIB appeared and gradually increased. Having attained the maximum at the seventh cycle of light-dark transition, it turned to a gradual decline. Because in the CO_2-free air the net photosynthetic CO_2 fixation was functionally blocked and the pool of depleted intermediates in the Calvin cycle could not be generated *via* the normal photosynthetic carbon assimilation, it could be speculated that the CO_2 released during the PIB was probably derived from leaf reserves of the fixed carbon, from which the pool of depleted intermediates in Calvin cycle could be partially restored.

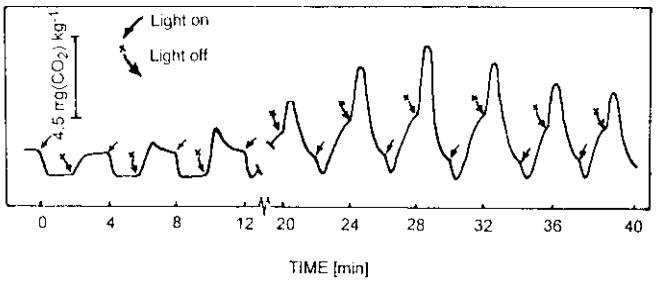

Fig. 2. The long-term CO_2 PIB by a darkened wheat leaf in the CO_2-free air over a period of alternate light-dark transitions. The potted wheat plant was kept in the dark for 24 h, and then the flag leaf was inserted in the leaf chamber flushed with the CO_2-free air. The PIB was monitored following initiation of the alternate light-dark transitions.

Light induction of CO_2 PIB in the darkened leaves: In order to investigate the dependence of CO_2 exchange in the CO_2-free air on leaf reserves of the fixed carbon, we kept the potted wheat plants in the dark for various periods. This darkening resulted in significant differences of the storage photosynthates contents (starch and sucrose) as depicted in Fig. 3. The cycle times of the light-dark transitions required

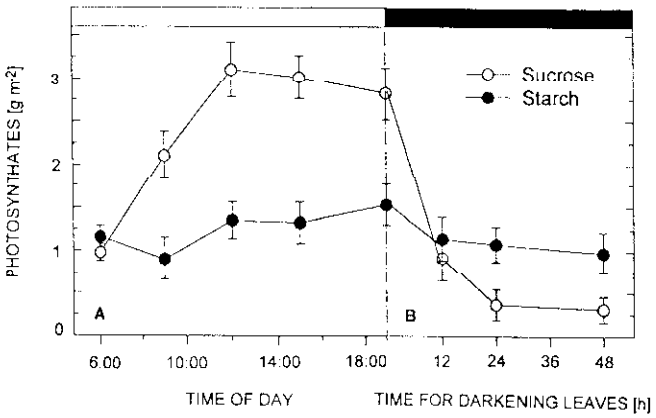

Fig. 3. Daily changes of sucrose and starch levels in the attached wheat leaves during the sunny day under the field condition (*A*), and their depletion over the extended dark period indoors (*B*).

for radiant energy induction of PIB or CO_2 evolution among the darkened wheat plants were different and largely dominated by the content of leaf reserves of storage

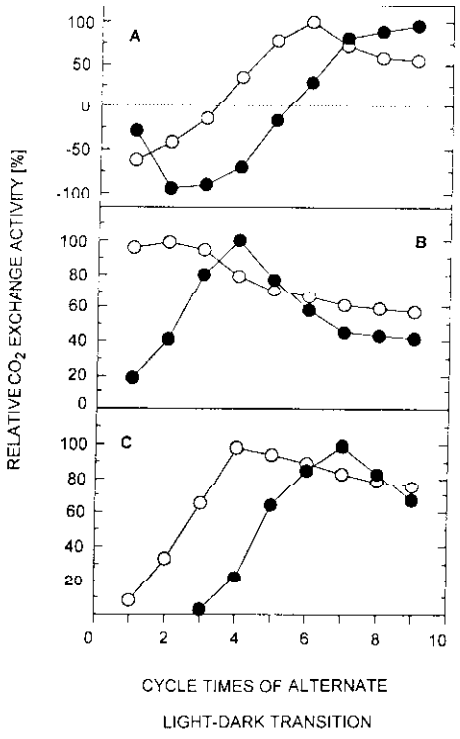

Fig. 4. Dynamic changes of the maximum capacity of CO_2 evolution (*A*), the maximum net uptake of respiratory CO_2 (*B*) in the light, and CO_2 PIB (*C*) in 8-h-darkened (○) or 32-h-darkened (●) wheat leaves in the CO_2-free air over the inductive period of alternate light (2 min)-dark (2 min) transitions. See Fig. 1 and the text for details.

photosynthates. For example, for an 8-h-darkened leaf, a detectable PIB could be easily elicited by only one cycle of light (2 min)-dark (2 min) transition (Fig. 4), while for a 32-h-darkened one, no significant PIB was monitored until the fourth cycle of alternate light-dark transitions. In addition, an obvious PIB in the 24-h-darkened leaves could be induced by a 10 min irradiation not only in the normal air [350 mg(CO_2) kg^{-1}, 21 % O_2], but also in CO_2-free air (21 % O_2), and even in CO_2-free gas mixture with a low oxygen concentration (2 % O_2). Thus the PIB induction in the darkened leaf may essentially depend neither on the new photosynthetic CO_2 fixation, nor on the photorespiratory carbon oxidation. Only radiant energy is crucial, although the underlying mechanisms remain to be elucidated.

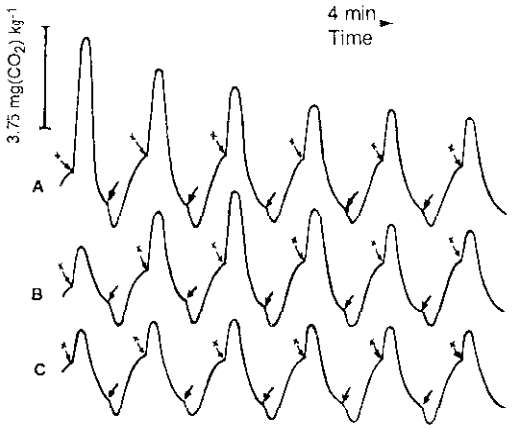

Fig. 5. The long-term CO_2 PIB over the period of alternate light-dark transitions by a 24-h-darkened wheat leaf. The darkened leaf was irradiated for 10 min either in the normal air (*A*), or in the CO_2-free air (21 % O_2, *B*), or in the CO_2-free gas mixture containing 2 % O_2 (*C*); then the air stream was quickly switched to the CO_2-free air. The PIB was initiated by continuing alternate light-dark transitions as shown by the arrows (also see Fig. 2).

Additional evidence applying to the role of radiant energy in the induction of PIB is provided in Fig. 6. For a 32-h-darkened leaf, the induction of CO_2 evolution with

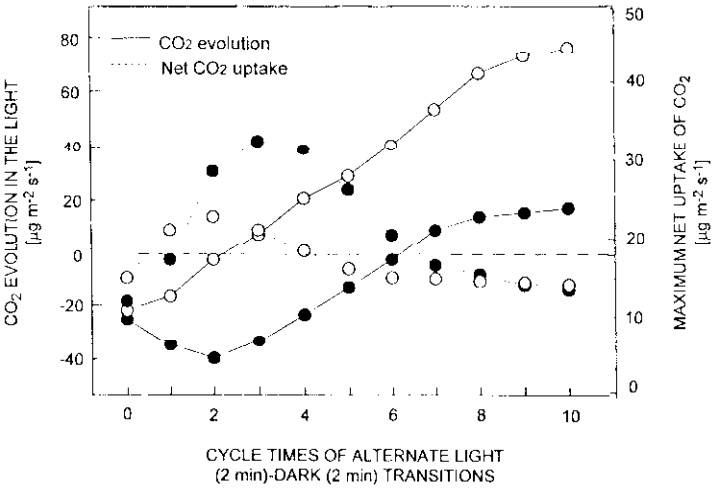

Fig. 6. The changes of maximum capacity of CO_2 evolution and the net uptake of respiratory CO_2 in the 32-h-darkened wheat leaves in CO_2-free air during induction with a high irradiance (850 μmol m^{-2} s^{-1}, ○) or a weak irradiance (90 μmol m^{-2} s^{-1}, ●).

a high irradiance (850 μmol m^{-2} s^{-1}) required 3 cycles of light-dark transitions, while with a low irradiance (90 μmol m^{-2} s^{-1}) 7 cycles were needed. The concomitant

changes of net uptake of respiratory CO_2 showed that the stimulative effect of strong irradiance on the induction of CO_2 evolution could not be simply ascribed to enhanced photosynthetic carbon fixation. Alternative mechanism was to be taken into consideration. According to Neuhaus *et al.* (1995), starch degradation in isolated intact amyloplasts incubated with ATP greatly facilitates the accumulation of DHAP and hexose-P. Both are highly available for further metabolism in the Calvin cycle. Based on the results of Fig. 6, and also in conjunction with the change of PIB rate during light induction (Figs. 4 and 5), the role of radiant energy in the induction of PIB in darkened leaves seems to initiate and accelerate mobilization of storage photosynthates (*e.g.*, starch or sucrose), which, in turn, facilitates the establishment of the availability of intermediates' pool in the Calvin cycle.

Gas exchange between air environment and plant leaves is accomplished mainly *via* stomata (Cowan 1977). Opening of stomata performs a light-induced process when the leaf darkened for an extended period is irradiated (Knapp and Smith 1990). Considering possible effects of stomata behaviour during the light induction on the pattern of CO_2 exchange in a darkened leaf, the change of stomata conductance in the 24-h-darkened wheat leaf was monitored during saturating irradiation of the leaf (850 μmol m^{-2} s^{-1}). The result presented in Fig.7 demonstrates a progressive increase in stomata conductance over the induction period. However, since the CO_2 efflux and CO_2 influx are equally dependent upon stomata behavior, such a change in stomata conductance cannot entirely explain the distinct characteristics of CO_2 exchange observed with darkened leaves during the induction irradiance.

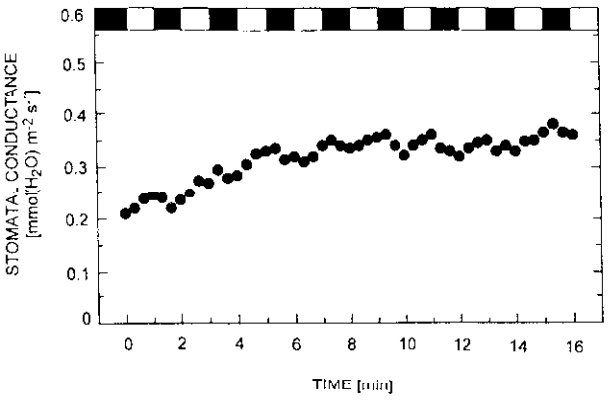

Fig. 7. Light-induced changes in stomata conductance in the 32-h-darkened wheat leaf over continuously alternated light-dark transitions shown by the broken bar at the top of the figure.

Effects of oxygen level in CO_2-free gases on CO_2 PIB: The enhancement by O_2 of the photorespiratory CO_2 evolution in C_3 plant leaves has been documented by Dochlert *et al.* (1979), Hitz and Stewart (1980), and Peterson (1983b). Our observations (Fig. 8) revealed that not only the maximum rate of PIB, but also the time of its maintainance was affected to a large extent by O_2 concentration which was further superimposed by the dependence on the leaf reserves of storage photosynthates. For example, with a wheat leaf pre-irradiated for 30 min in normal air [350 mg(CO_2) kg^{-1}, 21 % O_2], the detectable PIB could last for more than 1 h in CO_2-free gas mixture containing 15 % O_2 (Fig. 8*a*), while in the CO_2-free gas mixture containing

8 % O_2 it sustained for less than 35 min (Fig. 8*b*). If the leaf was flushed over with CO_2-free air (21 % O_2) in the light for only *ca.* 5 min, then the PIB could restore and maintain, with a much higher rate, for an additional extended period (Fig. 8*b*). Unlike the pre-irradiated ones, the 32-h-darkened leaf having been irradiated for 10 min in the normal air performed the PIB which sustained only *ca.* 15 min with alternate light-dark transitions in CO_2-free gas mixture containing 15 % O_2 (Fig. 8, *inset*).

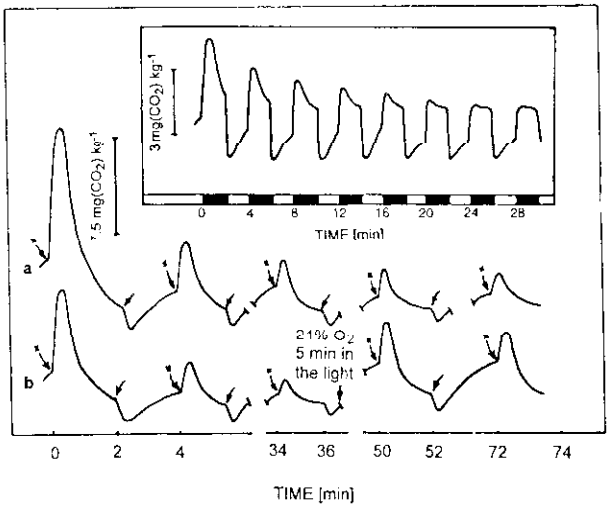

Fig. 8. Influence of oxygen level on long-term CO_2 PIB in wheat leaves. For the pre-photosynthesized leaf, CO_2 PIB was monitored in CO_2-free gas mixture containing 15 % O_2 (*a*) or 8 % O_2 (*b*). For comparison, CO_2 PIB in CO_2-free gas mixture (15 % O_2) by a 32-h-darkened wheat leaf is shown as *inset*. In order to record the detectable PIB, the darkened leaf was irradiated for 10 min in the normal air prior to the measurement.

Obviously, the initial released CO_2 in CO_2-free gas mostly originated from the newly fixed carbon *via* photosynthesis during the pre-irradiation of leaves in the normal air. Alternatively, the long-term CO_2 evolution could probably derive from storage photosynthates *via* a certain process of mobilization. Similar speculation was made by Fock *et al.* (1979) and Pärnik and Keerberg (1995) based on the changes of specific activity of photorespiratory released $^{14}CO_2$, and also by Somerville and Ogren (1980) based on the gas exchange analysis of photorespiratory mutants in *Arabidopsis thaliana*. Our results further showed that this mobilization was radiant energy-dependent, and stimulated by irradiance and O_2 level.

Effects of various chemical reagents on CO_2 exchange in CO_2-free air: In order to distinguish the contribution of metabolism of saccharides *via* the dark respiration *versus* that *via* the PCO-mediated process to long-term CO_2 evolution in the CO_2-free air, the effects of various inhibitors and reagents on CO_2 exchange in wheat leaves in the CO_2-free air were investigated (Table 1). P_i stimulates the degradation of starch in chloroplasts, resulting in a higher content of triose-phosphates in the Calvin cycle (Stitt *et al.* 1980). During our experiment, P_i enhanced both the PIB and net uptake of respiratory CO_2 in the light, confirming that both CO_2 uptake and PIB depended upon the availability of photosynthetic intermediates in the Calvin cycle. No significant effect of iodoacetic acid, the inhibitor of dark respiration, was observed on all parameters of CO_2 exchange except that CO_2 release in the dark showed some depression (values not shown). Also the PIB, CO_2 evolution in the

Table 1. Effects of P_i, N-ethylmaleimide (NEM), iodoacetic acid, and DCMU on CO_2 exchange [$\mu g(CO_2)$ m^{-2} s^{-1}] in wheat leaves in CO_2-free air.

Addition [mol m^{-3}]	Net max. CO_2 uptake	CO_2 PIB	CO_2 evolution in light
control	14.7 ± 0.5	54.2 ± 4.7	20.8 ± 2.0
P_i, 20	23.0 ± 1.1	65.0 ± 7.3	14.7 ± 0.9
NEM, 10	12.3 ± 0.3	33.7 ± 3.2	14.5 ± 0.6
Iodoacetic acid, 2×10^{-3}	15.3 ± 0.2	51.7 ± 6.2	19.8 ± 1.0
DCMU, 2×10^{-3}	12.8 ± 0.4	41.0 ± 4.2	15.7 ± 0.9

light and, to a less extent, the maximum net uptake of respiratory CO_2 in the CO_2-free air were suppressed by DCMU. Similar inhibition was observed in the treatment of NEM, an inhibitor of amylase in chloroplast (Levi and Gibbs 1984, Caspar *et al.* 1991). These results indicate that (*1*) degradation of starch in photosynthetic tissue is functionally involved in a long-term CO_2 evolution in the CO_2-free air in the light; and (*2*) the saccharide metabolism *via* dark respiration is neither an essential, nor a limiting step for long-term CO_2 exchanges in the CO_2-free air.

Discussion

In spite of numerous reports on CO_2 PIB both in the normal atmosphere and in CO_2-free air (Decker 1959, Yemm 1969, Zelitch 1980, Cornic and Gaudillère 1981, Marek *et al.* 1995), most investigations dealing with the PIB in CO_2-free air were merely accomplished with an attempt at quantifying the magnitude of photorespiration more accurately (Peterson 1983a, Palovský and Hák 1988, Sharkey 1988). Still there is a lack of available information about the probable influence of adverse environmental factors (*e.g.*, CO_2 deficiency under a high irradiance, water stress, *etc.*) upon PCR and PCO and, in turn, their corresponding responses upon the stresses. In this study, we found that wheat leaves, whatever pre-irradiated or darkened, showed a distinguishable PIB, which sustained, with a substantial rate much higher than that of the dark respiration, for an extended period (more than 2 h for the pre-irradiated leaf) with continuously alternate light-dark transitions in CO_2-free air (Figs. 1 and 2). Although our observations were accomplished under an extreme CO_2 deficiency, *i.e.* in the CO_2-free air, we believe that this long-term CO_2 efflux is actually an indicator of alternative carbon metabolism in photosynthetic apparatus as a response to photoinhibitory condition. The photosynthetic carbon metabolism is a process highly regulated by the availability of CO_2 *in vivo* (Wardlaw 1982) and/or the photosystem 2 excitation pressure (Savitch *et al.* 1996). Indeed, Fox and Geiger (1984), for example, have found that even a moderate reduction of CO_2 concentration from the ambient to 125 000 cm^3 m^{-1} initiates a shift of starch metabolism from net accumulation into net degradation. Following this finding and also based on the results of our observations, we propose that the mobilization of storage photosynthates and the subsequent PCO-mediated carbon recycle in C_3 plants

under CO_2 deficiency are physiologically-significant adaptive processes which occur *in vivo* once the PCR less matches photochemical reactions of photosynthesis.

In CO_2-free air, the photosynthetic carbon assimilation is virtually blocked with respect to the net carbon fixation. Consequently, no net carbon acquirement occurs in the leaves. Under such conditions, a certain amount of released CO_2 can be reassimilated by the irradiated leaf (Powles and Osmond 1978, Krause and Cornic 1987). Nevertheless, a majority of CO_2 generated by PCO was released out of the leaf. Thus, the underlying mechanism for long-term CO_2 efflux in CO_2-free air could be eventually attributed to the leaf reserve of the fixed carbon and its mobilization. We postulate that certain process(es) relevant probably to storage photosynthates (starch or sucrose) mobilization function under such conditions. As a result, complementary carbon supply may return the Calvin cycle, supporting long-term CO_2 efflux in CO_2-free air. To address this, the degradation of starch in the isolated intact spinach mesophyll cells in CO_2-free medium with a high irradiance was characterized (Xiong *et al.*, unpublished).

Whereas the degradation and the export of storage photosynthates occur simultaneously with their synthesis during photosynthesis (Stitt and Heldt 1981), their modification, especially the regulation of starch degradation in the photosynthetic tissue, remain unclear (Stitt and Steup 1985, Beck and Ziegler 1989). Specifically, much less is known about the putative influence of CO_2 deficiency superimposed with strong irradiance upon the mobilization of storage photosynthates (Savitch *et al.* 1996). We found that a long-term CO_2 efflux in the CO_2-free air was largely stimulated by increasing irradiance (Fig. 6) and O_2 concentration (Figs. 5 and 8), but unaffected by the dark respiration inhibitor, and suppressed by DCMU and NEM (Table 1). Assuming that the long-term CO_2 efflux in the CO_2-free air is associated with the mobilization of storage photosynthates, it may be concluded that this process is radiant energy-dependent, PCO-mediated, and distinct from the dark respiration. A conclusive elucidation of the biochemical mechanism requires an assay of starch degradative enzyme activities isolated from the C_3 plant leaves exposed to a high irradiance in CO_2-free air, which is in progress.

The observations of CO_2 exchange in the CO_2-free air have revealed the complex interaction between CO_2 uptake and CO_2 evolution in the light. For example, in the CO_2-free air, the pre-irradiated leaf preferentially displayed a substantial activity of PIB or CO_2 release in the light. Concomitantly, a very weak net uptake of respiratory CO_2 was monitored which could only sustain for about 15–25 s, followed by a shift from net CO_2 uptake into CO_2 evolution (see Fig. 1, *inset, c-d*). In contrast, the darkened leaf exhibited undetectable PIB or CO_2 evolution during the initial period of alternate light-dark transitions. Rather, a relatively strong net uptake of respiratory CO_2 was revealed (Figs. 2, 4, and 6). The biochemical mechanism underlying such a shift in CO_2 exchange pattern from the preferential CO_2 uptake towards to CO_2 evolution in the light remains to be established.

While PCO-mediated carbon cycle under CO_2 deficiency was generally viewed as a "futile cycle" or a "maintaining operation" with respect to its function in leaf carbon gain (Krause *et al.* 1978, Krause 1988), its possible physiological role in protecting the C_3 photosynthetic apparatus from photoinhibition is noteworthy. The maintaining

operation of PCO in the CO_2-free air will inevitably result in a loss of leaf reserves of the fixed carbon. However, as a return, complementary recovery of the pool of intermediates in the Calvin cycle will keep it from over-exhaustion, essential for a quick initiation of PCR when the conditions become favourable for photosynthetic carbon fixation. More important, excessive assimilatory energy can be at least partly dissipated *via* such PCO-mediated carbon recycling (Krause *et al.* 1978, Wu *et al.* 1991). Our results indicated that the mobilization of storage photosynthates was probably even more effective in dissipating excessive energy and relieving over-reduction of the photosynthetic electron transfer chain. This was not only because it provided a complementary carbon supply for a long-term PCO running under CO_2 deficiency (Figs. 1 and 2), but also the process was much irradiance-dependent (Fig. 6, Table 1). An early study demonstrated that the ^{14}C-efflux in isolated tobacco mesophyll cells was effected by an energy-dependent conversion of non-mobile assimilates into a mobile form (Scorer 1984). We believe that under a CO_2 deficiency, such PCO-mediated carbon recycling supported by mobilization of storage photosynthates might be one of the protective strategies used for the benefit of the photosynthetic apparatus of C_3 plants when alleviating the photoinhibitory damage *in vivo*.

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