Components of CO_2 exchange in leaves of C_3 species with different ability of starch accumulation

H. IVANOVA, H. KEERBERG, T. PÄRNIK, and O. KEERBERG+

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, 51014 Tartu, Estonia

Abstract

Using a radiogasometric method the rates of photorespiratory and respiratory decarboxylations of primary and stored photosynthates in the leaves of two groups of C_3 species, differing in the ability of starch accumulation, were determined. One group included starch-accumulating (SA) species with rates of starch synthesis on the average 38 % the rate of photosynthesis [*Solanum tuberosum* L., *Arabidopsis thaliana* (L.) Heynh, *Helianthus annuus* L., and *Plantago lanceolata* L.]. The second group represented starch-deficient (SD) species with rates of starch synthesis less than 8 % the rate of photosynthesis (*Secale cereale* L., *Triticum aestivum* L., *Hordeum vulgare* L., and *Poa trivialis* L.). In SA species the rate of respiration in the dark was significantly higher than in SD species. No differences were found in the rates of photosynthesis, photorespiration, and respiration under irradiation. Thus, the degree of inhibition of respiration by irradiation was in SA species higher than in SD species. It is concluded that starch does not provide substrates for respiratory and photorespiratory decarboxylations in irradiated photosynthesizing leaves.

Additional key words: intracellular decarboxylation; photorespiration; photosynthesis; respiration; starch-accumulating species; starch-deficient species; starch degradation.

Introduction

Respiration and photorespiration depend on substrates formed in the reactions of the photosynthetic carbon metabolism. These compounds may be divided into two groups, primary photosynthates and stored photosynthates. Primary photosynthates are metabolites of current photosynthesis with a turnover halftime in the order of minutes. Stored photosynthates are the end products of photosynthesis (for the most part starch, sucrose, and/or fructans) accumulating during the day in photosynthesizing leaves. The turnover halftime of these compounds is in the order of hours or tens of hours. Both primary and stored photosynthates can be used as substrates for photorespiratory as well for respiratory decarboxylations under irradiation (Pärnik and Keerberg 1995). However, methods commonly used in studies of photorespiration and respiration under irradiation (see review by Hunt 2003) do not distinguish between these two groups of substrates. Nevertheless, knowledge of the contribution of primary and stored photosynthates to different types of decarboxylation reaction is important for understanding the interrelations of photosynthesis, photorespiration, and respiration in irradiated leaves, and in connection with it, what is the substrate basis of the inhibition of respiration by irradiation, largely variable in different species (Pärnik *et al.* 2002, Hurry *et al.* 2005). Both the primary and stored photosynthates are involved as substrates of respiration under irradiation while only stored photosynthates could be consumed in respiration in the dark. A special problem is related to substrate contribution of stored sucrose and starch to photorespiratory and respiratory decarboxylations under irradiation. In the dark both sucrose and starch provide substrates for respiratory decarboxylations. However, it may not to be valid for the consumption of these compounds under irradiation. Starch degradation is suppressed by irradiation as demonstrated in pulse chase labelling experiments with leaves of pea (Kruger *et al.* 1983), sugar beet (Fox and Geiger 1984), and *Arabidopsis* (Zeeman and ap Rees 1999, Keerberg *et al.* 2005).

As an aid in elucidating the carbon source of decarboxylations under irradiation we designed a combined radiogasometric method enabling the determination of the rates of respiration and intracellular decarboxylation under steady-state photosynthesis, distinguishing between photorespiratory and respiratory CO_2 fluxes, and the estimation of the contribution of primary and stored

Received 2 November 2006, *accepted* 31 August 2007.
+Author for correspondence; fax: +372 6511142, e-mail: olav@ebi.ee
Acknowledgements: We highly appreciate the skilful technical assistance of Mrs. Kaarin Laas. This work was supported by grants from the Estonian Science Foundation (projects 4173 and 5989).

photosynthates as substrates for photorespiration and respiration (Pärnik and Keerberg 1995, Bykova *et al.* 2005). The method has been applied in studies of respiratory and photorespiratory metabolism in a number of C_3 species. In this paper we present the data on respiratory and photorespiratory decarboxylations in two groups of C_3 species differing in the ability of starch accumulation in the leaves. *Solanum tuberosum*, *Arabidopsis thaliana*, *Helianthus annuus*, and *Plantago lanceolata* were considered as starch-accumulating (SA) species with high rate of starch synthesis while *Secale cereale*, *Triticum aestivum*, *Hordeum vulgare*, and *Poa trivialis* represented starch-deficient (SD) species with low rate of starch synthesis. The aim of this study was to establish how the extent of starch accumulation affects the rates and ratio of decarboxylation components under irradiation and in the dark. In SA species the rate of respiration in the dark was significantly higher than in SD species while no differences were detected in the rates of respiration under irradiation. Thus, the degree of light inhibition of respiration was in SA species higher than in SD species, the fact suggesting that starch degradation and consumption in photorespiratory and respiratory decarboxylations were suppressed by irradiation.

Materials and methods

Plants of eight C_3 species, *Solanum tuberosum* L., *Arabidopsis thaliana* (L.) Heynh, *Helianthus annuus* L., *Plantago lanceolata* L., *Secale cereale* L., *Triticum aestivum* L., *Hordeum vulgare* L., and *Poa trivialis* L. were grown in soil under combined irradiation of high-pressure sodium vapour lamp *LU400/HO/T/40NG* (*Lucalox*, Hungary) and high-pressure mercury vapour fluorescent lamp *LRF 250W E40* (*Polamp*, Poland), or under fluorescent tubes *BS-30* (*Saransk Factory*, Russia) at following conditions: irradiance 250–300 μmol m^{-2} s^{-1}, light/dark period 16/8 h (8/16 h for *A. thaliana*), and day/night temperature 22/16 °C. All measurements were carried out with fully expanded attached leaves on the second half of photoperiod (3–8 h after switching on the light) under environmental conditions indicated in descriptions of corresponding experiments.

Radiogasometric measurements of CO_2 exchange: Photosynthetic and respiratory CO_2 fluxes were determined under steady-state photosynthesis using a radiogasometric method (Pärnik and Keerberg 1995, Bykova *et al.* 2005). The measurements of CO_2 exchange were performed in a rapidly operating multi-channel exposure system described by Pärnik *et al.* (1987). The amount of $^{14}CO_2$ evolved from leaves, previously exposed to $^{14}CO_2$, was measured in a closed gas system by means of Geiger-Müller counters. The components of $^{12}CO_2$ exchange, *i.e.* net photosynthesis (P_N), respiration in the dark (R_D), and CO_2 efflux into CO_2-free atmosphere were measured in the open gas system with *LI-6262* CO_2/H_2O analyzer (*LI-COR*, Lincoln, NE, USA). The rate of transpiration (E), stomatal conductance (g_s), and internal concentration of CO_2 (C_i) were determined as described by Laisk and Oja (1998).

The radiogasometric method enables to determine intracellular rates of photorespiration and respiration in irradiated leaves under steady-state photosynthesis and distinguish primary and stored photosynthates as substrates of photorespiratory and respiratory decarboxylations. Primary and stored photosynthates are distinguished on the basis of their different labelling kinetics during exposure of leaves to $^{14}CO_2$. Pools of primary photosynthates (such as the metabolites of the reductive pentose phosphate cycle and of the glycolate cycle, intermediates of starch and sucrose synthesis and of glycolysis) are saturated with ^{14}C by 10–15 min of exposure to $^{14}CO_2$ (Ivanova *et al.* 1993). On the contrary, long-term exposures (2–3 h) are required to achieve any measurable level of the specific radioactivity of $^{14}CO_2$ evolved from stored photosynthates (starch, sucrose, fructans, vacuolar acids). Discrimination between photorespiration and respiration is based on their different dependence on oxygen concentration assuming that photorespiration is linearly dependent on oxygen concentration up to 210 dm^3 m^{-3} while respiration becomes saturated with oxygen at about 15 dm^3 m^{-3}. Determination of intracellular re-fixation of respiratory CO_2 is based on the assumption that probability of re-fixation of $^{14}CO_2$ evolved inside the cell is close to zero when measurements are performed at very high concentrations (30 dm^3 m^{-3}) of $^{12}CO_2$.

Determination of the rate of starch synthesis was made from the kinetics of its labelling under steady-state photosynthesis in the medium of $^{14}CO_2$. Leaves were exposed to $^{14}CO_2$ for 5, 20, 60, 120, 360, and 600 s and killed in boiling ethanol. The soluble compounds were extracted twice in boiling 80 % (v/v) ethanol and once in boiling acidified 96 % ethanol. The leaf material remaining after the extraction was incubated in a solution of α-amylase [*Boehringer*, Germany, 200 g m^{-3} (m/v) in sodium citrate buffer, pH 6.8] for 48 h at 35 °C and the radioactivity of hydrolyzed starch determined in the liquid scintillation counter *LS 100C* (*Beckman*, USA). From the values of radioactivity and of the specific radioactivity of $^{14}CO_2$ fed to leaves the amounts of carbon incorporated into starch were calculated and plotted against the duration of exposure of leaves to $^{14}CO_2$ (see Fig. 1 as an example). The experimental points were fitted to function

$$^{14}C(t) = rt + c[\exp(-rt/c) - 1] \quad (1)$$

describing time dependence of the amount of label incorporated into the end product of a linear reaction

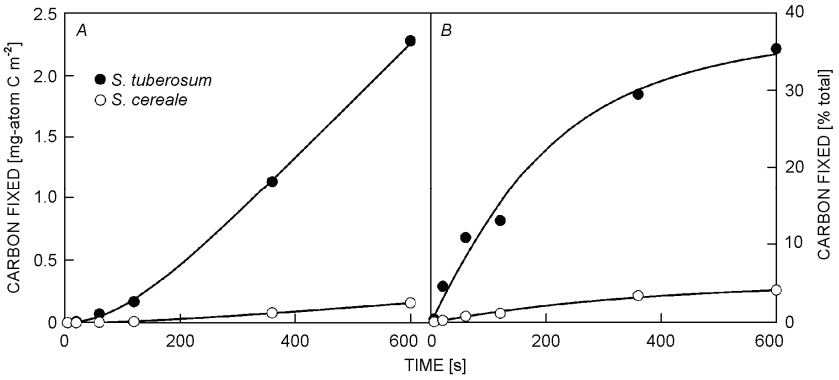

Fig. 1. The absolute (*A*) and relative (*B*) radioactivity of starch as a function of the duration of exposure of leaves to $^{14}CO_2$ under steady-state photosynthesis. Symbols represent the experimental points, curves are the theoretical values calculated from Eq. 1 after its fitting to experimental points by means of nonlinear regression procedure.

chain fed under steady-state conditions with labelled substrate (Keerberg and Viil 1988). In Eq. 1 $^{14}C(t)$ is the amount of carbon incorporated into starch during feeding the leaves with $^{14}CO_2$ for t s, parameter r is the molar rate of carbon incorporation into starch (the steady-state rate of starch synthesis), and parameter c is the total pool of precursors of starch in its biosynthetic pathway. The values of r and c were determined by means of nonlinear regression procedure.

Results

Rates of starch synthesis in leaves of different C_3 species: The rates of starch synthesis were calculated from its labelling curves in leaves irradiated for different periods in the medium of $^{14}CO_2$ (see Materials and methods). Measurements were performed on the 2nd half of photoperiod under irradiances saturating photosynthesis. The species studied may be divided into two contrasting groups differing in the rates of starch synthesis. In the first group, including forbs *S. tuberosum*, *H. annuus*, *A. thaliana*, and *Plantago lanceolata*, 28–44 % of the photosynthetically fixed carbon was incorporated into starch (on the average 38 % of the rate of photosynthesis). In the second group, that includes grasses *H. vulgare*, *T. aestivum*, *S. cereale*, and *Poa trivialis*, less than 8 % of fixed carbon was used for starch synthesis (on the average 6.8 % of the rate of photosynthesis). In the further presentation we shall refer to the species of the first group as starch-accumulating (SA) species and to the species of the second group as starch-deficient (SD) species.

Rates of photorespiratory and respiratory decarboxylations in C_3 species with different rates of starch synthesis: Table 1 presents the results of radiogasometric measurements of the rates of true photosynthesis (P_T), the rates of total (R), photorespiratory (R_P), and respiratory (R_R) decarboxylations under irradiation, and the rates of respiration in the dark (R_D) in leaves of different C_3 species. P_T was defined as the sum of the rates of net photosynthesis (P_N) and of CO_2 evolution from leaves under irradiation (R_L). R, R_P, and R_R are the real intracellular rates of decarboxylations taking into account the re-fixation of respiratory CO_2. R_D was measured 30 min after switching off the light. From the molar rates the ratios of the rates of decarboxylation components to the rates of true photosynthesis (R/P_T, R_P/P_T, and R_R/P_T), and the ratio of the rate of respiratory decarboxylation under irradiation to the rate of respiration in the dark (R_R/R_D) were calculated. Measurements were performed at irradiances saturating photosynthesis (in the range 450–1 000 μmol m^{-2} s^{-1} in different experiments).

Measurements were carried out in three replications on different leaves or set of leaves and means of the replications were calculated. Characteristics of CO_2 exchange in leaves of *S. tuberosum*, *H. annuus*, *T. aestivum*, and *H. vulgare* were determined in two and of *S. cereale* in four independent experiments. In Tables 1 and 2 the mean values of data derived from these experiments (means of experiments) are presented. Limits of standard errors are not shown, the differences in gas exchange parameters of species belonging into the same group were not considered. For the both groups of species the mean values±SE of the rates and ratios of carbon fluxes were calculated. The significance (p<0.05) of differences between means of data from experiments with SA and SD species was estimated with two-sample t-test.

The average rates of P_T were similar in SA and SD species. In all species the prevailing decarboxylation mechanism was photorespiratory decarboxylation with the rate four to seven times exceeding the rate of respiratory decarboxylation. No significant differences between SA and SD species were found in the molar rates of total, photorespiratory, and respiratory decarboxylations and in their ratios to the rate of P_T under irradiation. The relative rates of total decarboxylation (R/P_T) varied in the range 17–21 %, rates of photorespiratory decarboxylations (R_P/P_T) in the range 15–18 %, and rates of respiratory decarboxylations (R_R/P_T) in the range 2–4 % of R_T.

In SA species R_D was about 1.5 times higher and the

ratio R_R/R_D two-fold lower than in SD species. Thus, in SA species irradiation suppressed respiration four times while in SD species only two-fold suppression was found. The different degree of suppression by irradiation of respiration in SA and SD species may be completely attributed to the different rates of respiration in the dark as the rates of respiratory decarboxylations under irradiation (R_R) were almost the same in both groups of studied species suggesting that consumption of starch as substrate of decarboxylations may be suppressed or blocked under irradiation.

Table 1. Rate of true photosynthesis (P_T), rates of total (R), photorespiratory (R_P), and respiratory (R_R) decarboxylations under irradiation and rate of respiration in the dark (R_D) [μmol(CO_2) m^{-2} s^{-1}] and their ratios [%] in leaves of starch-accumulating species (*upper part of the Table*) and in starch-deficient species (*lower part of the Table*). The components of CO_2 exchange were determined by a radiogasometric method (see Materials and methods) under steady-state photosynthesis at 360–380 cm^3 m^{-3} CO_2, 210 dm^3 m^{-3} O_2, and 450–1 000 μmol m^{-2} s^{-1} PFD. [1]Mean of three replications in one experiment. [2]Mean of two experiments, both in three replications. [4]Mean of four experiments, each in three replications. [a]Values in one column are significantly different (p<0.05 established by two-sample t-test).

Species	P_T	R	R_P	R_R	R_D	R/P_T	R_P/P_T	R_R/P_T	R_R/R_D
Solanum tuberosum[2]	18.63	3.24	2.89	0.35	1.29	17.2	15.4	1.9	26.7
Arabidopsis thaliana[1]	8.65	1.63	1.48	0.15	0.99	18.8	17.1	1.7	15.2
Helianthus annuus[2]	23.58	4.53	3.90	0.63	2.10	19.2	16.0	2.7	30.1
Plantago lanceolata[1]	14.33	2.42	2.12	0.30	1.12	16.9	14.8	2.1	26.8
Mean±SE	16.30±3.17	2.95±0.62	2.60±0.52	0.36±0.10	1.37[a]±0.25	18.0±0.6	15.8±0.5	2.1±0.2	24.7[a]±3.3
Secale cereale[4]	13.06	2.59	2.08	0.51	0.85	21.1	16.8	3.9	60.1
Triticum aestivum[2]	16.64	3.32	2.85	0.48	0.87	21.2	18.3	2.9	56.8
Hordeum vulgare[2]	12.75	2.45	2.16	0.29	0.52	19.3	17.0	2.3	54.5
Poa trivialis[1]	15.90	3.19	2.82	0.37	1.03	20.1	17.7	2.3	35.9
Mean±SE	14.59±0.99	2.89±0.21	2.47±0.21	0.41±0.05	0.82[a]±0.11	20.4±0.5	17.5±0.4	2.9±0.4	51.8[a]±5.4

Table 2. The total rates of decarboxylation of primary (R_A) and stored (R_S) photosynthates and the rates of their consumption in photorespiratory (R_{PA} and R_{PS}, respectively) and respiratory (R_{RA} and R_{RS}, respectively) decarboxylations [μmol(CO_2) m^{-2} s^{-1}] and their ratios [%] under irradiation in leaves of starch-accumulating species (*upper part of the Table*) and in starch-deficient species (*lower part of the Table*). The experimental conditions and the values of P_T and R_D as in Table 1. [1]Mean of three replications in one experiment. [2]Mean of two experiments, both in three replications. [4]Mean of four experiments, each in three replications. [a]Values in one column are significantly different (p<0.05 established by two-sample t-test).

Species	R_A	R_{PA}	R_{RA}	R_S	R_{PS}	R_{RS}	R_A/P_T	R_S/R_D	R_{RS}/R_D
Solanum tuberosum[2]	2.79	2.55	0.24	0.45	0.35	0.11	14.8	34.9	8.0
Arabidopsis thaliana[1]	1.12	1.05	0.07	0.51	0.43	0.08	13.0	51.5	8.1
Helianthus annuus[2]	3.87	3.32	0.55	0.26	0.18	0.08	16.0	11.9	4.2
Plantago lanceolata[1]	2.16	1.94	0.22	0.26	0.18	0.08	15.1	23.2	7.1
Mean±SE	2.48±0.57	2.21±0.48	0.27±0.10	0.37±0.06	0.28±0.06	0.09[a]±0.01	14.7±0.6	30.4[a]±8.5	6.9[a]±0.9
Secale cereale[4]	2.19	1.81	0.38	0.40	0.27	0.13	17.3	49.3	15.4
Triticum aestivum[2]	2.35	2.04	0.31	0.97	0.80	0.17	14.5	130.9	19.8
Hordeum vulgare[2]	1.51	1.46	0.05	0.94	0.70	0.24	11.8	182.0	45.2
Poa trivialis[1]	2.76	2.51	0.25	0.43	0.31	0.12	17.4	41.8	11.7
Mean±SE	2.20±0.26	1.85±0.22	0.25±0.07	0.69±0.16	0.52±0.13	0.17[a]±0.03	15.3±1.3	101.0[a]±33.7	23.0[a]±7.6

Substrates of photorespiratory and respiratory decarboxylations in C_3 species with different rates of starch synthesis: The substrates for photorespiratory and respiratory decarboxylations under irradiation are primary photosynthates (products of current photosynthesis) and stored photosynthates (products of previous photosynthesis). The prevailing substrates of decarboxylations under irradiation were primary photosynthates. The average rate of consumption of primary photosynthates (R_A) exceeded that of stored photosynthates (R_S) in SA species six times and in SD species three times (Table 2). Primary photosynthates were used mainly in photorespiratory decarboxylations (86 % the total rate). The rate of decarboxylation of primary photosynthates (R_A/P_T) made 13–17 % of the rate of P_T. Stored photosynthates were also used in both types of decarboxylations, preferably in photorespiration.

No large differences between SA and SD species were

detected in molar rates of decarboxylation components. Interestingly, the rates of consumption of stored photosynthates in decarboxylations were in SA species even lower than in SD species. Significant differences between SA and SD species were found in the ratios of decarboxylation of stored photosynthates under irradiation to the rate of respiration in the dark (R_S/R_D and R_{RS}/R_D). In the dark only stored photosynthates can be used in respiratory decarboxylations (there are no primary photosynthates and no photorespiration). The corresponding component under irradiation is R_{RS}. In SD species irradiation suppressed the respiratory decarboxylation of stored photosynthates on average four times while in SA species up to 15-fold suppression was found (compare values of R_{RS} and R_D in Tables 2 and 1). The ratio R_{RS}/R_D was significantly higher in SD species compared to SA species. The ratio of the total rate of (respiratory plus photorespiratory) decarboxylation of stored photosynthates under irradiation to the rate of respiration in the dark (R_S/R_D) was also higher in SD species. In SD species the rate of decarboxylation of stored photosynthates (R_S) was almost equal with the rate of respiration in the dark (R_D) while in SA species R_S was about three-fold lower than R_D. The differences of these ratios were derived from the higher R_D and from the lower rates of respiratory decarboxylation of stored photosynthates under irradiation in SA species compared to SD species (see Tables 1 and 2). The latter may be explained by the higher content of soluble stored photosynthates in SD species. The lower rates of decarboxylation of stored photosynthates in SA species indicate that products of starch breakdown did not compensate the lower content of soluble photosynthates, the fact pointing to the possibility that starch was not metabolized under irradiation.

Discussion

Species with different ability of starch accumulation: SA and SD species were distinguished on the basis of the steady-state rates of starch synthesis in photosynthesizing leaves. This approach was preferred to the commonly used measurements of starch content in plant leaves. The latter is largely variable during diurnal cycle. In grasses studied the rates of starch synthesis were five to seven times lower than in forbs. The low capacity of starch accumulation seems to be a common feature of grasses. Reviewing the literature data, Cairns *et al.* (2002) found that in temperate grasses the rates of starch accumulation were 5–50 fold and net starch contents two to five times lower than in a large variety of SA species surveyed. However, the C_3 temperate members of the Poales including winter rye, wheat, barley, and *Poa trivialis* are capable of producing and accumulating large quantities of fructans (Cairns *et al.* 2000).

Photosynthetic and respiratory CO_2-exchange in species with different rates of starch synthesis: In all species studied the prevailing mechanism of CO_2 production under irradiation was photorespiration. The rates of photorespiratory decarboxylations four to seven times exceeded the rates of respiratory decarboxylations (Table 1). The prevailing substrates of decarboxylation reactions were primary photosynthates. The contribution of stored photosynthates to decarboxylations was 15–30 % (Table 2). About 75 % of stored photosynthates were consumed in photorespiratory decarboxylations with the rate constituting 10–20 % of the total rate of photorespiration (Tables 1 and 2). Consumption of stored products of photosynthesis in photorespiration has been earlier suggested by Goldsworthy (1970). Mahon *et al.* (1974) established that after 15 min exposure of sunflower leaves to $^{14}CO_2$ the specific radioactivities of 3-phosphoglyceric acid, glycine, and serine were significantly lower than the specific radioactivity of the $^{14}CO_2$ originally fed to the leaves. These findings suggest that besides photosynthesis there must be an additional flow of unlabelled carbon into the reductive pentose phosphate cycle. At low concentrations, near to the CO_2 compensation concentration (where most of the gasometric measurements of respiration are performed), the substrates of photorespiration must be derived from stored photosynthates (Stitt *et al.* 1985). In these conditions contribution of primary photosynthates is low as the rate of net photosynthesis is close to zero.

No significant differences between SA and SD species were found in the rates of photosynthesis, photorespiration, and respiration under irradiation (Table 1). However, the rate of respiration in the dark was in SA species significantly higher than in SD species. It may be explained by the enhanced availability of substrates in SA species where large quantities of starch may be involved in respiration. A correlation between the concentration of starch and the rate of respiration in the night has been found in leaves of pepper (Grange 1985), soybean (Mullen and Koller 1988), and a *sex4* mutant of *Arabidopsis* with repressed endoamylase (Zeeman and ap Rees 1999). In wheat leaves the R_D was also dependent on the content of starch, the latter modified by duration of irradiation of leaves at different concentrations of CO_2 (Azcón-Bieto and Osmond 1983). The rate of starch degradation was relatively constant during the night (Zeeman and ap Rees 1999, Smith *et al.* 2005). The rate is adjusted to a value enabling consumption during the night of whole starch accumulated in leaves by the end of day. Thus the rate of starch degradation is determined by its content at the beginning of dark period. This is consistent with our data on higher R_D in SA species. However, the mechanism of this phenomenon is not known.

In SA species the extent of inhibition of respiration by irradiation was significantly higher than in SD species

(Table 1). This difference between SA and SD species may partly explain the large variability of literature data on the extent of inhibition of respiration by irradiation in different species (Atkin *et al.* 2000, Hurry *et al.* 2005). The suppression of respiration under photorespiratory conditions has been explained by the change of phosphorylation status of mitochondrial pyruvate dehydrogenase resulting in the loss of its activity (Tovar-Mendez *et al.* 2003). Functioning of the partial TCA cycle under irradiation has also been proposed to contribute to a decrease in respiratory decarboxylations (Hurry *et al.* 2005). Under irradiation, isocitrate dehydrogenase is suppressed by increased redox levels of the mitochondrial pools of NAD^+ and $NADP^+$ derived from photorespiration (Igamberdiev and Gardeström 2003). The main product of the partial TCA cycle under irradiation is citrate exported from mitochondria to the cytosol (Hanning and Heldt 1993). This would decrease decarboxylations linked to respiration.

Suppression of starch degradation in photosynthesizing leaves: In SA species the R_D was significantly higher than in SD species while no differences were found in R_L (Table 1). The consumption of stored photosynthates in decarboxylation reactions under irradiation was in SA species slower than in SD species (Table 2). These two facts suggest that under irradiation starch may not be involved in supplying the substrates for respiratory and photorespiratory decarboxylations. Thus, the differences in the extent of inhibition of respiration by irradiation in SA and SD species may be explained by the suppression of starch degradation in photosynthesizing leaves.

Inhibition of the degradation of ^{14}C-labelled starch under irradiation has been observed in leaves of pea (Kruger *et al.* 1983), sugar beet (Fox and Geiger 1984), and *Arabidopsis* of the wild type and of *sex4* mutant (Zeeman and ap Rees 1999, Zeeman *et al.* 2002). Degradation of starch is suppressed only in photosynthesizing leaves, the inhibition is eliminated in CO_2-free air under irradiation as demonstrated in experiments with leaves of sugar beet (Fox and Geiger 1984, 1986), spinach (Stitt *et al.* 1985), and *Arabidopsis* (Keerberg *et al.* 2005). The inhibitory signal, initiated at low irradiances, is derived from or mediated by the reactions of photosynthetic carbon metabolism.

The mechanisms of starch degradation are not fully understood. Numerous enzymes that could participate in starch breakdown are present in leaves but the relative importance of each has not been determined. Recently Zeeman *et al.* (2004) and Smith *et al*. (2005) proposed a pathway of starch breakdown in *Arabidopsis*. According to this scheme soluble glucans are released from starch granule by glucan, water dikinase (GWD), phosphoglucan, water dikinase (PWD), and/or α-amylase. Cleavage of linear glucans is catalyzed mainly by β-amylase. Formed in this reaction maltose is transported into the cytosol *via* specific maltose transporter. Maltose is considered as the main product of starch degradation, formation of glucose and glucose-1-P has minor relative importance. However, the mechanisms of light-dark regulation of starch degradation remain unclear. The suppression of starch degradation in photosynthesizing leaves has been explained by the reduction of the activity of participating enzymes induced by the changes of pH (Pongratz and Beck 1978, Okita and Preiss 1980), by changes of energy charge, ATP/ADP ratio, and the redox state of electron transport chain (Gfeller and Gibbs 1984, 1985, Walters *et al.* 2004), and by changes of the phosphate status of chloroplasts (Usuda and Shimogawara 1991, Qiu and Israel 1994, Zeeman *et al.* 2004, Smith *et al.* 2005).

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