S15-002

Contribution of primary and stored photosynthates to photorespiration and respiration in the light and in the dark

O Keerberg, H Ivanova, H Keerberg, T Pärnik

Institute of Experimental Biology at the Estonian Agricultural University, 76902 Harku, Estonia. olav@ebi.ee

Keywords: carbon metabolism, respiration, photorespiration, primary photosynthates, stored photosynthates

Introduction

Substrates of respiration and photorespiration are compounds formed in the reactions of the photosynthetic carbon metabolism. These compounds may by divided into two groups, primary photosynthates and stored photosynthates. Primary photosynthates are products of current photosynthesis with a turnover half-time in the order of minutes. They include the metabolites of the reductive pentose phosphate cycle, of the glycolate cycle, intermediates of starch and sucrose synthesis and glycolysis. During the exposure of leaves to $^{14}CO_2$ these compounds are saturated with ^{14}C within 10 min (Ivanova et al., 1993). Stored photosynthates are the end products of photosynthesis accumulating during the day in photosynthesizing leaves. These metabolites are characterized with turnover halftime in the order of hours and include the compounds such as starch, sucrose and vacuolar acids. Long-term exposure (2 to 3 h) of leaves to $^{14}CO_2$ is required to achieve any measurable level of the specific radioactivity of carbon in these compounds. It has been shown that primary and stored photosynthates are used as substrates of both respiration and photorespiration (Pärnik and Keerberg, 1995). In this paper we present data on the rates and ratio of consumption of primary and stored photosynthates in decarboxylation reactions in the light and in the dark. Six C_3-species with different ability of starch synthesis has been studied.

Materials and methods

Plant material

All measurements were carried out on fully expanded attached leaves of *Nicotiana tabacum, Solanum tuberosum, Arabidopsis thaliana, Hordeum vulgare, Triticum aestivum* and *Secale cereale.* Plants (except *A. thaliana)* were grown in soil under fluorescent and high pressure sodium lamps at 250-300 µmol m^{-2} s^{-1}, 16h/8h day/night, temperature 25°/20°C. The growth irradiance for *A. thaliana* was 150 µmol m^{-2} s^{-1}, photoperiod 8h/16h.

Measurement of CO_2 exchange

Photosynthetic and respiratory CO_2 fluxes were determined under steady-state photosynthesis at normal environmental conditions (350 µL L^{-1} CO_2, 25°C, saturating light) by means of a radiogasometric method (Pärnik and Keerberg, 1995). Method enables to distinguish respiratory carbon fluxes from primary and stored photosynthates on the basis of different labeling kinetics of these two groups of compounds in leaves exposed to ^{14}CO2. To determine the rate of $^{14}CO_2$ evolution from the primary photosynthates, leaves were preilluminated in 350 µL L^{-1} $^{12}CO_2$ until a stationary rate of photosynthesis was established. Leaves were then exposed to 350 µL L^{-1} $^{14}CO_2$ for 10-15 min to saturate the primary photosynthates with ^{14}C and the leaf chamber was then cleaned for 4 s with 300 µL L^{-1} $^{12}CO_2$. Efflux of $^{14}CO_2$ was recorded in 350 µL L^{-1} $^{12}CO_2$. From the initial slope of $^{14}CO_2$ evolution curve and the specific radioactivity of $^{14}CO_2$ fed to leaves the steady-state rate of CO_2 evolution from primary photosynthates was calculated. To distinguish between photorespiration and respiration measurements were carried out in parallel at 210 and 15 mL L^{-1} O_2 assuming that photorespiratory decarboxylation is linearly dependent on oxygen concentration at least up to 210 mL L^{-1} while respiratory decarboxylation becomes saturated with oxygen at about 15 mL L^{-1}. The initial rate of $^{14}CO_2$ evolution was measured also at a very high $^{12}CO_2$ concentration (30mL L^{-1}) to find the rates of intracellular decarboxylation and of intrafoliar reassimilation.

To determine the rate of $^{14}CO_2$ evolution from stored photosynthates leaves were exposed to 350 µL L^{-1} $^{14}CO_2$ for 4 h and were then illuminated in 350 µL L^{-1} $^{12}CO_2$ for 30 min to eliminate the ^{14}C from pools of primary photosynthates. The efflux of $^{14}CO_2$ from leaves was then measured at 350 µL L^{-1} $^{12}CO_2$ and at 210 or 15 mL L^{-1} O_2 in the light and in the dark. Dark measurements were performed after 10-15 min the light was switched off. Efflux of $^{14}CO_2$ and $^{12}CO_2$ was also measured in CO_2-free air to determine the specific radioactivity of CO_2 derived from stored photosynthates. From the initial slopes of $^{14}CO_2$ evolution curves and the specific radioactivity of evolved CO_2 the rates of respiratory and photorespiratory decarboxylation of stored photosynthates were calculated.

The method enables to determine the rates of respiratory CO_2 evolution and intracellular decarboxylation under steady-state photosynthesis. Four components of respiration in the light according to the substrates and mechanisms of decarboxylation reactions could be distinguished: (1) photorespiratory decarboxylation of primary photosynthates, (2) photorespiratory decarboxylation of stored photosynthates, (3) respiratory decarboxylation of primary photosynthates and (4) respiratory decarboxylation of stored photosynthates.

Measurement of starch radioactivity

After exposure of leaves to $^{14}CO_2$ the soluble compounds were extracted with hot ethanol or 6% perchloric acid. The remaining material was incubated in solution of amylase (250 mg L^{-1} in sodium citrate buffer) for 48 h at 35°C and the radioactivity of the hydrolysed starch was determined. Rates of starch synthesis were calculated from its labeling kinetics according to procedure described by Keerberg and Pärnik (1998).

Results

The species studied may be divided into two groups: “starch” plants (tobacco, potato, Arabidopsis) where the rate of starch synthesis exceeds 35% the rate of true photosynthesis and cereals (barley, wheat, winter rye) where less than 8% of photosynthetically fixed carbon is incorporated into starch (Table 1).

The relative rates of total decarboxylation in the light and contribution of stored and primary photosynthates to decarboxylation in different species are shown in Table 2.

Table 1. Relative rates of starch synthesis in photosynthesizing leaves of different C_3-species (per cent true photosynthesis)

Species	Rate of synthesis
Nicotiana tabacum	43±0.8
Solanum tuberosum	44±1.0
Arabidopsis thaliana	38±2.4
Hordeum vulgare	7.3±0.2
Triticum aestivum	7.0±0.2
Secale cereale	5.6±0.2

Table 2. Relative rates of total intracellular decarboxylation in the light and contribution of primary and stored photosynthates to decarboxylation in leaves of different C_3-species (per cent true photosynthesis)

Species	Decarboxylation		
	Total	Primary photosynthates	Stored photosynthates
Nicotiana tabacum	33.5±3.3	19.3±2.5	14.2±2.2
Solanum tuberosum	16.7±1.2	14.1±1.0	2.6±0.7
Arabidopsis thaliana	23.0±1.3	15.9±1.1	7.1±0.6
Hordeum vulgare	30.8±4.6	15.7±3.6	15.1±3.0
Triticum aestivum	29.6±3.4	16.2±0.1	13.4±3.4
Secale cereale	35.2±4.1	28.7±4.0	6.5±0.7

In species tested the rate of decarboxylation varies in the range from 17% (potato) to 35% (rye) the rate of true photosynthesis. In all species, particularly in potato and winter rye, the prevailing substrates of decarboxylation are primary photosynthates. No distinct differences in these characteristics were found between “starch” plants and cereals.

In Fig.1. the rates of decarboxylation of stored photosynthates in the dark and in the light are compared. In the light stored photosynthates are used not only in respiratory but also and prevailingly in photorespiratory decarboxylations. In leaves of cereals the total rate of photorespiratory and respiratory decarboxylations in the light was higher than in

the dark while in “starch”plants , on the contrary, stored photosynthates were consumed with higher rate in the dark.

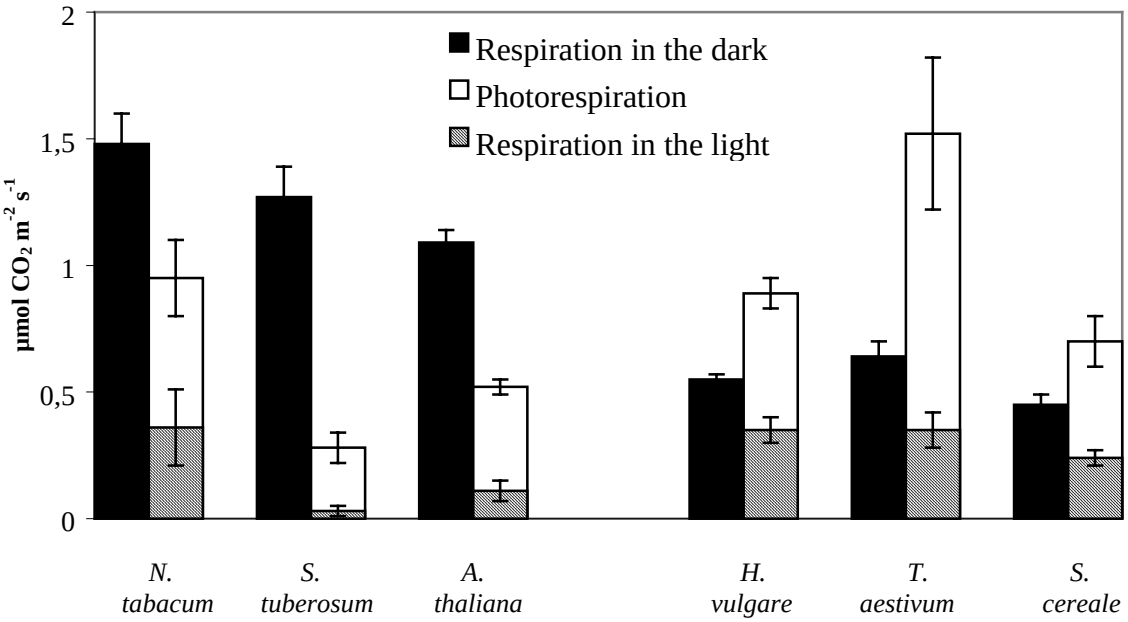

Fig.1. Rates of respiratory and photorespiratory decarboxylation of stored photosynthates in the dark and in the light in leaves of different C_3-species.

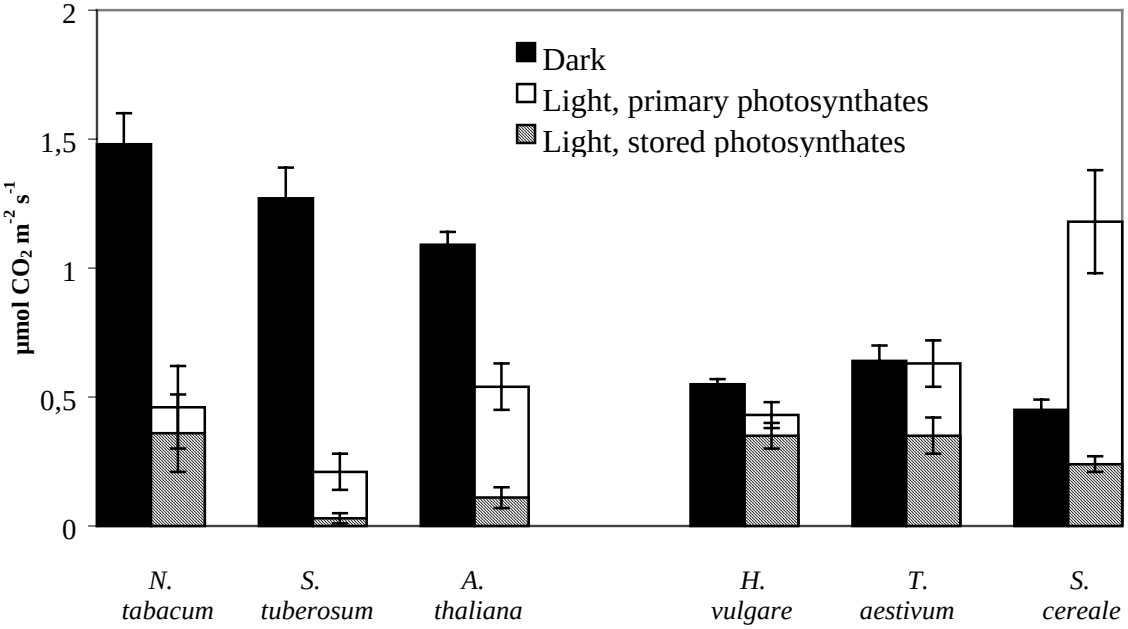

Fig.2. Rates of respiratory decarboxylation of stored and primary photosynthates in the dark and in the light in leaves of different C_3-species.

Fig.2. shows the rates of respiratory decarboxylation of primary and stored photosynthates in the dark and in the light. In the light primary photosynthates are consumed not only in photorespiration but also in respiration. The only component of respiration operative in the dark is the respiratory decarboxylation of stored photosynthates. As seen from the figure in all species this component was suppressed by light, more severely in "starch" plants. In "starch" plants the total rate of respiration as sum of decarboxylations of primary and stored photosynthates was also suppressed by light while no definite light inhibition was found in cereals.

The suppression of decarboxylation of stored photosynthates and respiration by light in "starch" plants but not in cereals suggests that consumption of starch in respiratory decarboxylations is blocked by light. To check this assumption we performed an experiment where the catabolism of labeled photosynthates was followed in the light and in the dark. Two intact plants of *A. thaliana* were exposed to $^{14}CO_2$ in the light for 75 min. After that half of leaves from both plants was removed and radioactivity of soluble compounds and starch determined. One of the plants with rest of leaves was then transferred into the dark, the another plant was left in the light. After 3h storage in nonradioactive medium the radioactivity of the same compounds in leaves and roots of both plants was determined. The decrease of the radioactivity of soluble compounds and starch during the exposure in nonradioactive air characterize the extent of their utilization in respiratory and transport processes. The results of the experiment are given in Table 3.

Table 3. Radioactivity of labeled soluble compounds and starch in leaves of *A. thaliana* after 3h light and dark period in nonradioactive medium (per cent initial radioactivity)

	Soluble compounds	Starch
Light	73.8±2.8	104.8±3.9
Dark	86.4±2.0	63.1±2.8

Data represent mean values of 4 experiments

In the light the radioactivity of soluble compounds decreased 26% while in the dark the decrease was only 14%. It means that utilization of soluble compounds was not suppressed but even stimulated by light. Surprisingly no loss of radioactivity of starch was detected in the light. Its radioactivity even increased slightly. The same result was obtained in a preliminary experiment with winter rye where radioactivity of starch increased in the light 10% (data not shown). However in the dark a significant decrease, 37%, of the radioactivity of starch was detected (Table 3). The loss of the radioactivity of photosynthates may be attributed mainly to their utilization in respiratory processes because in roots was found only 2.4% (after light period) and 5.6% (after dark period) of the initial radioactivity. Thus the only substrates of respiratory decarboxylations in the light are soluble compounds, degradation of starch is blocked by light.

Discussion

The fact that stored photosynthates are used not only in respiration but also in photorespiration suggests that products of their degradation must be transferred into reductive pentose phosphate cycle and, via this cycle, into the glycolate cycle. This

suggestion is in agreement with data of Mahon *et al.* (1974) who established that, after 15 min exposure of sunflower leaves to $^{14}CO_2$ specific radioactivities of PGA, glycine and serine were significantly lower than the specific radioactivity of $^{14}CO_2$ fed to leaves, the fact indicating a flow of carbon into the reductive pentose phosphate cycle from some unlabelled source other than ambient CO_2. Thus the reductive pentose phosphate cycle is fed from two sources: CO_2 from air and products of degradation of stored photosynthates. However, according to our data, only soluble stored photosynthates but not starch are used in photorespiratory decarboxylations in the light. The prevailing soluble compound accumulating in leaves of *A. thaliana* is sucrose.. About 40% of photosynthetically fixed carbon is incorporated into sucrose (unpublished data). To be transferred into reductive pentose phosphate cycle products of sucrose degradation must be transported from cytosol to chloroplasts. We assume that most likely it occurs via triose phosphate/PGA translocator. Once being incorporated into reductive pentose phosphate cycle carbon derived from sucrose may be used not only in photorespiration but also in synthesis of starch, the fact explaining a slight increase of starch radioactivity in the light (Table 3).

The mechanisms that regulate starch degradation remain unclear. Gfeller and Gibbs (1984) demonstrated that in cells of green alga *Chlamydomonas reinhardtii* the breakdown of starch was suppressed by light up to 4 times compared to the dark rate. However Stitt and Heldt (1981) found that in illuminated spinach chloroplasts starch was degraded with the rate comparable with the rate of its synthesis. The process was stimulated by orthophosphate, a compound required in phosphorolytic degradation of starch. Reduced availability of orthophosphate in chloroplasts of intact leaves may be one of reasons why the starch breakdown was blocked by light in our experiments.

Acknowledgments

This work was supported by the grants of the Estonian Science Foundation (Projects No 2197 and 4173).

References

Gfeller RP, Gibbs M (1984) *Plant Physiology* **75,** 212-218.
Ivanova H, Keerberg O, Pärnik T (1993) *Proceedings of the Estonian Academy of Sciences. Chemistry* **42,** 185-197.
Keerberg O, Pärnik T (1998) *BioThermoKinetics in the Post Genomic Era* 303-306.
Mahon JD, Fock H, Canvin DT (1974) *Planta* **120,**125-134.
Pärnik T, Keerberg O (1995) *Journal of Experimental Botany* **46,** 1439-1447.
Stitt M, Heldt HW (1981) *Biochimica et Biophysica Acta* **638,** 1-11.