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Respiration by the oxidative pentose phosphate pathway in chloroplasts responds to atmospheric CO_2 concentration

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Abstract

Despite significant research efforts, the question of whether rising atmospheric CO_2 concentrations (C_a) affect leaf respiration remains unanswered. Here, I reanalyse published hydrogen isotope abundances in starch glucose of sunflower leaves. I report that, as C_a increases from 450 to 1500 ppm, respiration by the oxidative pentose phosphate pathway in chloroplasts increases from 0 to ≈ 5% relative to net carbon assimilation. This is consistent with known regulatory properties of the pathway. Summarising recent reports of metabolic fluxes in plant leaves, a picture emerges in which mitochondrial processes are distinctly less important for overall respiration than the oxidative pentose phosphate pathways in chloroplasts and the cytosol. Regulatory properties of these pathways are consistent with observations of lower-than-expected stimulations of photosynthesis by increasing C_a. Reported advances in understanding leaf respiratory mechanisms may enable modelling and prediction of respiration effects (*inter alia*) on biosphere-atmosphere CO_2 exchange and plant performance under climate change.

Introduction

Despite significant research efforts, the question of whether rising atmospheric CO_2 concentrations (C_a) affect leaf respiration remains unanswered (Gonzàlez-Meler *et al.*, 2004; Way *et al.*, 2015; Dusenge *et al.*, 2019). A large body of research conveys an entirely inconsistent picture including reports of both positive and negative responses. This may (*inter alia*) be due to methodological difficulties to disentangle overlapping CO_2 fluxes at the tissue level and an incomplete understanding of respiration at the metabolic level with a strong research focus on mitochondrial processes. Overall, leaf respiration has remained a major unknown from the metabolic to the Earth system level.

State-of-the-art isotope techniques enable analyses of specific metabolic fluxes (Ehlers *et al.*, 2015; Wieloch *et al.*, 2021b, 2022c; Xu *et al.*, 2022). Recently, we reported two deuterium (D) fractionation signals (i.e., systematic D variability) in starch glucose of sunflower leaves (Wieloch *et al.*, 2022a). A signal at glucose H^1 reflects hydrogen isotope fractionation by chloroplast glucose-6-phosphate dehydrogenase (G6PD) and associated flux through the oxidative pentose phosphate pathway (OPPP, Fig. 1A). This anaplerotic pathway feeds pentose phosphates into the Calvin-Benson cycle (CBC), supplies NADPH, and releases CO_2. Another signal at glucose H^2

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reflects hydrogen isotope fractionation by chloroplast phosphoglucose isomerase (PGI) and associated shifts of the reaction from kinetic to equilibrium conditions.

Previously, we investigated these processes in leaves of sunflowers raised over 7-8 weeks at C_a = 450 ppm (Wieloch *et al.*, 2022a). We reported evidence against anaplerotic flux under these conditions. However, moving the plants into a low C_a atmosphere for two days led to significant increases in δD_1 and δD_2 consistent with an increase in anaplerotic flux and a shift of the PGI reaction from kinetic to equilibrium conditions, respectively. Related fractionation signals were also found in the starch derivative tree-ring glucose under drought (Wieloch *et al.*, 2018, 2022b).

Here, I reanalyse our previously published data (Wieloch *et al.*, 2022a) to assess how metabolism behaves after moving the plants into a high C_a atmosphere for two days. I report that, as C_a increases from 450 to 1500 ppm, respiration by the OPPP in chloroplasts increases from 0 to ≈ 5% relative to net carbon assimilation. This is consistent with known regulatory properties of the pathway. Summarising recent reports of metabolic fluxes in plant leaves, a picture emerges in which mitochondrial processes are distinctly less important for overall respiration than the oxidative pentose phosphate pathways in chloroplasts and the cytosol. Regulatory properties of these pathways are consistent with observations of lower-than-expected stimulations of photosynthesis by increasing C_a. Reported advances in understanding leaf respiratory mechanisms may enable modelling and prediction of respiration effects (*inter alia*) on biosphere-atmosphere CO_2 exchange and plant performance under climate change.

Material and Methods

Here, I reanalyse published hydrogen isotope abundances in starch glucose of sunflower leaves (Wieloch *et al.*, 2022a). Isotope fractionations at glucose H^1 and H^2 are respectively expressed as

$$\delta D_1 = \frac{D_1}{D_{6S}} - 1 \quad (1)$$

and

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$$\delta D_2 = \frac{D_2}{D_{6R}} - 1 \quad (2)$$

where D_i denotes relative D abundances at specific glucose hydrogen positions. In these equations, the D abundances at glucose H^{6S} and H^{6R} are used as references because glucose H^1 and H^{6S} and H^2 and H^{6R} have the same precursors at the triose-phosphate level, and H^{6S} and H^{6R} are not modified in the starch biosynthesis pathway (Wieloch *et al.*, 2022a). In these notations, δD_1 increases above zero reflect increases in anaplerotic flux into the CBC while δD_2 increases from negative to positive values reflect shifts of the PGI reaction from being on the side of F6P over being at equilibrium to being on the side of G6P (Wieloch *et al.*, 2022a).

Results and Discussion

Anaplerotic flux responds to increasing C_a

In contrast to C_a = 450 ppm, δD_1 is significantly greater than zero under C_a = 700 and 1500 ppm (Fig. 1B; one-tailed one-sample t-test: $p<0.05$, n=5). This is consistent with anaplerotic flux into the CBC. By contrast, δD_2 exhibits low values of around -425‰ over the entire C_a range (Fig. 1C) indicating that the PGI reaction remains stably removed from equilibrium on the side of fructose 6-phosphat (F6P). The absence of a δD_2 response is remarkable because anaplerotic flux was proposed to be controlled at the level of PGI (Sharkey & Weise, 2016). Accordingly, we previously observed simultaneous shifts of δD_1 and δD_2 for C_a shifts below 450 ppm (Wieloch *et al.*, 2022a). Thus, the results suggest regulatory differences of the anaplerotic pathway for low and high C_a conditions.

In the light, chloroplast G6PD is inhibited by redox regulation via thioredoxin (Née *et al.*, 2009), yet inhibition may be reversed allosterically by increasing concentrations of glucose 6-phosphate (G6P) (Cossar *et al.*, 1984; Preiser *et al.*, 2019). At medium C_a, the PGI reaction in chloroplasts is strongly removed from equilibrium on the side of F6P resulting in low [G6P]/[F6P] ratios and G6P concentrations (Dietz, 1985; Gerhardt *et al.*, 1987; Kruckeberg *et al.*, 1989; Schleucher *et al.*, 1999). Low G6P concentrations are believed to restrict the anaplerotic flux (Sharkey & Weise, 2016). Towards low C_a, G6P concentrations increase more than F6P concentrations, i.e., the PGI reaction shifts towards equilibrium (Dietz, 1985). Towards high C_a, [G6P]/[F6P] ratios remain

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low, yet F6P and G6P concentrations both increase along with net carbon assimilation (Dietz, 1985). Thus, towards low C_a, G6P concentrations and anaplerotic flux increase due to regulation at PGI. By contrast, increases towards high C_a are not caused by regulation at PGI but probably by increases in net carbon assimilation and concomitantly increasing G6P concentrations.

Estimation of anaplerotic flux and associated respiration at high C_a

A previously published model describing hydrogen isotope fractionation by G6PD can be used to estimate anaplerotic flux into the CBC, associated respiration, and NADPH supply (Wieloch *et al.*, 2022a). At C_a = 700 ppm, ≈ 4% of the G6P entering the starch biosynthesis pathway is diverted into the anaplerotic pathway while it is ≈ 9% at 1500 ppm. Assuming 50% of all net assimilated carbon becomes starch (Sharkey *et al.*, 1985), anaplerotic respiration is ≈ 2% and ≈ 5% relative to net carbon assimilation at C_a = 700 and 1500 ppm, respectively (50/96*4, 50/91*9). These estimates are based on δD_1 signal strengths in starch glucose. At medium to high C_a, the PGI reaction is on the side of F6P (Fig. 1C) (Dietz, 1985). To a degree, this prevents conversion of G6P (the site of signal introduction) back to F6P. F6P may leave the starch biosynthesis pathway via transketolase causing signal washout (Wieloch *et al.*, 2022a). At low C_a, the PGI reaction is closer to or at equilibrium and signal washout can be expected to be significant (Wieloch *et al.*, 2022a). Thus, the G6PD fractionation model may significantly underestimate anaplerotic flux at low C_a, while high-C_a estimates can be expected to be closer to actual values.

Explanations for the lower-than-expected stimulation of photosynthesis by increasing C_a

Net carbon assimilation is stimulated by increasing C_a. However, measured increases usually fall short of theoretical increases calculated from rubisco kinetics. For instance, Rogers *et al.* (2004) reported that soybean leaves only achieve about half of their theoretical potential. Interestingly, these authors (among others) also reported significantly increased leaf glucose concentrations at high C_a which may indicate increased chloroplastic G6P concentrations, and anaplerotic flux. Similarly, Tjoelker *et al.* (2009) report a positive relationship between leaf sugar concentrations and respiration in pine needles. Thus, respiration by the anaplerotic pathway may, in part, explain offsets between measured and theoretical values of net carbon assimilation at increased C_a.

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Another part may be explained by respiration from the cytosolic OPPP. Primary control of flux through this pathway is exerted at G6PD. In the cytosol of Arabidopsis leaves, only G6PD5 was found (Wakao and Benning 2005). Its activity increases with increasing sugar availability (sugars tested: mannose, glucose, fructose, sucrose) through de novo enzyme synthesis (Hauschild and von Schaewen 2003). As C_a increases, so does net carbon assimilation and sugar availability (Lawlor and Fock 1977; Sánchez-Rodríguez et al. 1999). This can be expected to cause increasing cytosolic OPPP flux and associated respiration.

Increases of respiration by both OPPPs may be transient. Over time, plants subjected to higher C_a may increase their sink capacities and export increasing amounts of sugar from source to sink tissues. Decreasing leaf sugar levels may then result in reduced OPPP respiration in chloroplasts and the cytosol.

OPPP flux in chloroplasts introduces a δD_1 signal in starch (see above). Similarly, OPPP flux in the cytosol of leaves can be expected to introduce a δD_1 signal in the glucose and fructose moieties of sucrose. These signals will be recorded in tree-ring glucose. However, signal interpretation will be complicated by several processes including signal washout in chloroplasts (see above) and OPPP flux and triose phosphate cycling in the cytosol of tree-ring cells. Nevertheless, I believe these complications can be addressed and encourage the development of δD_1 signal analysis to retrieve information about leaf respiration and respiratory acclimation to increasing C_a from leaf, phloem, and tree-ring metabolites.

A paradigm shift in the field of leaf respiration

In the past, research of leaf respiration had a strong focus on mitochondrial processes. However, according to recent analyses of metabolic fluxes in leaves of *Arabidopsis thaliana* and *Camelina sativa*, mitochondrial respiration is relatively low (1 to 1.6% relative to net carbon assimilation) (Ma *et al.*, 2014; Xu *et al.*, 2022). By contrast, respiration by the cytosolic OPPP is 5% relative to net carbon assimilation in *Camelina sativa* leaves (Xu *et al.*, 2022). Similarly, in sunflower leaves, respiration by the OPPP in chloroplasts is relatively high under both high (see above) and low C_a (Wieloch *et al.*, 2021a, 2022a). These findings put OPPPs at the centre of leaf respiration.

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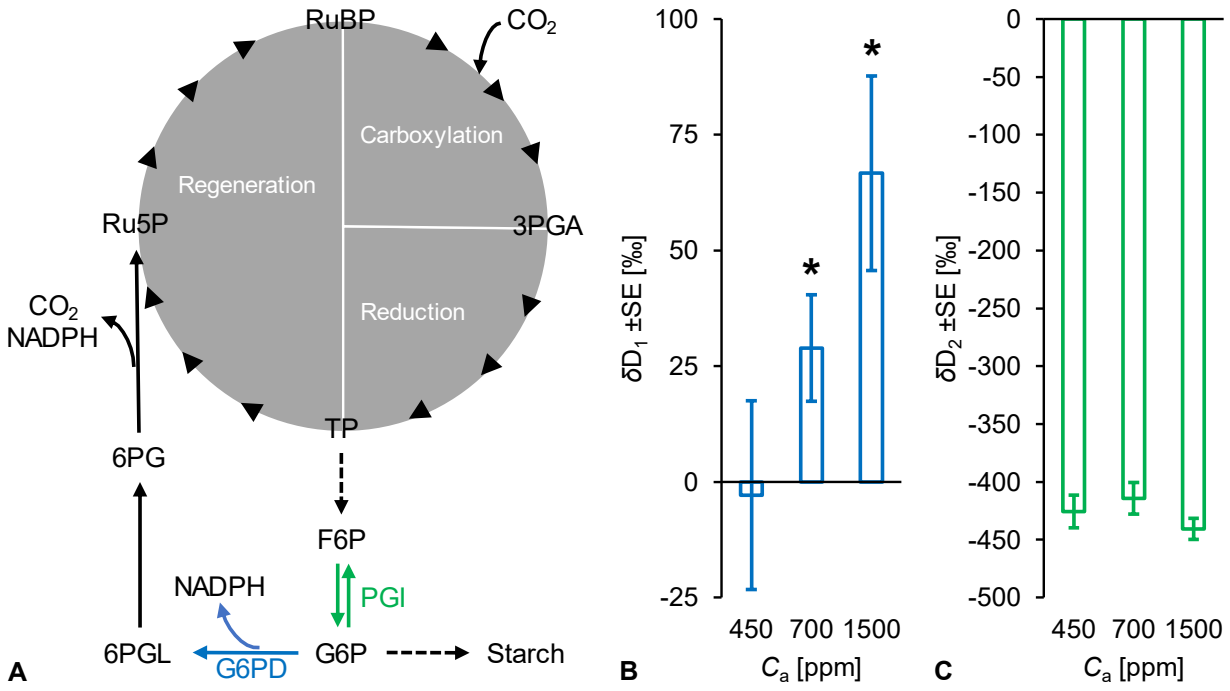

Figure 1 (**A**) Oxidative pentose phosphate pathway in chloroplasts carrying anaplerotic flux into the Calvin-Benson cycle (grey). Blue and green: Enzyme reactions which introduce D fractionation signals at glucose H^1 and H^2, respectively. Dashed arrows: Intermediate reactions not shown. Enzymes: G6PD, glucose-6-phosphate dehydrogenase; PGI, phosphoglucose isomerase. Metabolites: 3PGA, 3-phosphoglycerate; 6PG, 6-phosphogluconate; 6PGL, 6-phosphogluconolactone; F6P, fructose 6-phosphate; G6P, glucose 6-phosphate; NADPH, nicotinamide adenine dinucleotide phosphate; Ru5P, ribulose 5-phosphate; RuBP, ribulose 1,5-bisphosphate; TP, triose phosphates (glyceraldehyde 3-phosphate, dihydroxyacetone phosphate). Modified figure from Wieloch *et al.* (2022b). (**B, C**) Deuterium (D) abundance at glucose H^1 (blue bars), and H^2 (green bars) of *Helianthus annuus* leaf starch. Asterisks denote D abundances that are significantly greater than zero (one-tailed one-sample t-test: $p<0.05$, n=5). The plants were raised in chambers over 7 to 8 weeks at C_a = 450 ppm. After a day in darkness to drain the starch reserves, the plants were grown for two days at different levels of C_a (450, 700, 1500 ppm) corresponding to different levels of C_i (328, 531, 1365 ppm). Data expressed as $\delta D_1 = D_1/D_{6S}-1$ and $\delta D_2 = D_2/D_{6R}-1$ where D_i denotes relative D abundances at specific glucose hydrogen positions. D abundances at glucose H^{6S} and H^{6R} are used as references because glucose H^1 and H^{6S} and H^2 and H^{6R} have the same precursors at the triose-phosphate level, and H^{6S} and H^{6R} are not modified in the starch biosynthesis pathway (Wieloch *et al.*, 2022a).

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Data availability

The data supporting the findings of this study have been published previously (Wieloch *et al.*, 2022a).

Competing interest statement: The author declares no competing interests.

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